

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C12N 9/02, 15/64, 15/81, 15/82, C12P 7/64, C12N 5/16, A01K 67/00, A23L 1/00, A61K 31/202, 7/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 00/40705</b> <b>(43) International Publication Date:</b> 13 July 2000 (13.07.00)
<b>(21) International Application Number:</b> PCT/US99/31163 <b>(22) International Filing Date:</b> 29 December 1999 (29.12.99)  <b>(30) Priority Data:</b> 09/227,613 8 January 1999 (08.01.99) US  <b>(71) Applicant:</b> ABBOTT LABORATORIES [US/US]; CHAD-0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 6050 (US).  <b>(72) Inventors:</b> MUKERJI, Pradip; 1069 Arcaro Drive, Gahanna, OH 43230 (US). LEONARD, Amanda, E., Y.; 581 Shadewood Court, Gahanna, OH 43230 (US). HUANG, Yung-Sheng; 2462 Danvers Court, Upper Arlington, OH 43220 (US). PARKER-BARNES, Jennifer, M.; 1311 Belcross Drive, New Albany, OH 43054 (US).  <b>(74) Agents:</b> BECKER, Cheryl, L. et al.; Abbott Laboratories, CHAD/0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-6050 (US).		<b>(81) Designated States:</b> AU, BG, BR, CA, CN, CZ, HU, IL, JP, KR, MX, NO, NZ, PL, RO, SI, SK, TR, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> HUMAN DESATURASE GENE AND USES THEREOF  <b>(57) Abstract</b>  The subject invention relates to the identification of a gene involved in the desaturation of polyunsaturated fatty acids at carbon 5 (i.e., "human $\Delta 5$ -desaturase") and to uses thereof. In particular, human $\Delta 5$ -desaturase may be utilized, for example, in the conversion of dihomo- $\gamma$ - linolenic acid (DGLA) to arachidonic acid (AA) and in the conversion of 20:4n-3 to eicosapentaenoic acid (EPA). AA or polyunsaturated fatty acids produced therefrom may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## HUMAN DESATURASE GENE AND USES THEREOF

The subject application is a Continuation-In-Part of pending U.S. Patent Application Serial No. 09/227,613 filed on January 8, 1999, which is a Continuation-In-Part of pending International Application PCT/US98/07422 filed on April 10, 1998 (which designates the U.S.) which is a Continuation-In-Part of pending U.S. Patent Application Serial No. 08/833,610 filed on April 11, 1997, all of which are incorporated herein in their entirety by reference.

BACKGROUND OF THE INVENTIONTechnical Field

15

The subject invention relates to the identification and isolation of a gene that encodes an enzyme (i.e., human  $\Delta 5$ -desaturase) involved in the synthesis of polyunsaturated fatty acids and to uses thereof. In particular,  $\Delta 5$ -desaturase catalyzes the conversion of, for example, dihomo- $\gamma$ -linolenic acid (DGLA) to arachidonic acid (AA) and (n-3)-eicosatetraenoic acid (20:4n-3) to eicosapentaenoic acid (20:5n-3). The converted product may then be utilized as a substrate in the production of other polyunsaturated fatty acids (PUFAs). The product or other polyunsaturated fatty acids may be added to pharmaceutical compositions, nutritional composition, animal feeds as well as other products such as cosmetics.

Background Information

30

Desaturases are critical in the production of long-chain polyunsaturated fatty acids which have many important functions. For example, PUFAs are important components of the plasma membrane of a cell, where they are found in the form of phospholipids. They also serve as precursors to mammalian

prostacyclins, eicosanoids, leukotrienes and prostaglandins. Additionally, PUFAs are necessary for the proper development of the developing infant brain as well as for tissue formation and repair. In view of the biological significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, in an efficient manner.

A number of enzymes are involved in PUFA biosynthesis including  $\Delta 5$ -desaturase (see Figure 11). For example, elongase (elo) catalyzes the conversion of  $\gamma$ -linolenic acid (GLA) to dihomogamma-linolenic acid (DGLA) and of stearidonic acid (18:4n-3) to (n-3)-eicosatetraenoic acid (20:4n-3). Linoleic acid (LA, 18:2- $\Delta 9,12$  or 18:2n-6) is produced from oleic acid (18:1- $\Delta 9$ ) by a  $\Delta 12$ -desaturase. GLA (18:3- $\Delta 6,9,12$ ) is produced from linoleic acid by a  $\Delta 6$ -desaturase.

It must be noted that animals cannot desaturate beyond the  $\Delta 9$  position and therefore cannot convert oleic acid into linoleic acid. Likewise,  $\alpha$ -linolenic acid (ALA, 18:3- $\Delta 9,12,15$ ) cannot be synthesized by mammals. However,  $\alpha$ -linolenic acid can be converted to stearidonic acid (STA, 18:4- $\Delta 6,9,12,15$ ) by a  $\Delta 6$ -desaturase (see PCT publication WO 96/13591 and The Faseb Journal, Abstracts, Part I, Abstract 3093, page A532 (Experimental Biology 98, San Francisco, CA, April 18-22, 1998) see also U.S. Patent No. 5,552,306), followed by elongation to (n-3)-eicosatetraenoic acid (20:4- $\Delta 8,11,14,17$ ) in mammals and algae. This polyunsaturated fatty acid (i.e., 20:4- $\Delta 8,11,14,17$ ) can then be converted to eicosapentaenoic acid (EPA, 20:5- $\Delta 5,8,11,14,17$ ) by a  $\Delta 5$ -desaturase, such as that of the present invention. Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbon 12 (see PCT publication WO 94/11516 and U.S. Patent No. 5,443,974) and carbon 15 (see PCT



publication WO 93/11245). The major polyunsaturated fatty acid of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid or  $\alpha$ -linolenic acid. In view of these difficulties, it is of significant interest to  
5 isolate genes involved in PUFA synthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant, or animal system which can be altered to provide production of commercial quantities of one or more PUFAs. One of the most important long chain PUFAs, noted above,  
10 is arachidonic acid (AA). AA is found in filamentous fungi and can also be purified from mammalian tissues including the liver and adrenal glands. As noted above, AA production from dihomogamma-linolenic acid is catalyzed by a  $\Delta 5$ -desaturase. EPA is another important long-chain PUFA. EPA is found in fungi and also in  
15 marine oils. As noted above, EPA is produced from (n-3)-eicosatetraenoic acid and is catalyzed by a  $\Delta 5$ -desaturase.

In view of the above discussion, there is a definite need for the  $\Delta 5$ -desaturase enzyme, the gene encoding this enzyme, as well as recombinant methods of producing this enzyme.  
20 Additionally, a need exists for oils containing levels of PUFAs beyond those naturally present as well as those enriched in novel PUFAs. Such oils can only be made by isolation and expression of the  $\Delta 5$ -desaturase gene.

All U.S. patents and publications referred to herein are  
25 hereby incorporated in their entirety by reference.

#### SUMMARY OF THE INVENTION

The present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 50%  
30 of the nucleotide sequence shown in SEQ ID NO:1 (Figure 12).

The isolated nucleotide sequence may be represented by SEQ ID NO:1. These sequences may encode a functionally active desaturase which utilizes a polyunsaturated fatty acid as a substrate. The sequences may be derived from a mammal such as,  
5 for example, a human.

The present invention also includes purified proteins encoded by the nucleotide sequences referred to above. Additionally, the present invention includes a purified polypeptide which desaturates polyunsaturated fatty acids at  
10 carbon 5 and has at least about 50% amino acid similarity to the amino acid sequence of the purified proteins referred to directly above.

Furthermore, the present invention also encompasses a method of producing a human  $\Delta 5$ -desaturase. This method  
15 comprises the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:1 (Figure 12); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the  
20 human  $\Delta 5$ -desaturase. The host cell may be, for example, a eukaryotic cell or a prokaryotic cell. In particular, the prokaryotic cell may be, for example, E. coli, cyanobacteria or B. subtilis. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant cell or a fungal cell  
25 (e.g., a yeast cell such as Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. or Pichia spp.).

Additionally, the present invention also encompasses a  
30 vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 12) operably linked to b) a promoter. The invention also includes a host cell comprising this vector.

The host cell may be, for example, a eukaryotic cell or a prokaryotic cell. Suitable eukaryotic cells and prokaryotic cells are as defined above.

Moreover, the present invention also includes a plant cell,  
5 plant or plant tissue comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, selected from the group consisting of AA and EPA.  
10 The invention also includes one or more plant oils or acids expressed by the above plant cell, plant or plant tissue.

Additionally, the present invention also encompasses a transgenic plant comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production  
15 of a polyunsaturated fatty acid in seeds of the transgenic plant.

Also, the invention includes a mammalian cell comprising the above vector wherein expression of the nucleotide sequence of the vector results in production of altered levels of AA or  
20 EPA when the cell is grown in a culture media comprising a fatty acid selected from the group consisting of an essential fatty acid, LA and ALA.

It should also be noted that the present invention encompasses a transgenic, non-human mammal whose genome  
25 comprises a DNA sequence encoding a human  $\Delta 5$ -desaturase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:1 (Figure 12). Additionally, the present invention includes a fluid (e.g., milk) produced by the transgenic, non-human mammal wherein the fluid comprises a detectable level of  
30 at least human  $\Delta 5$ -desaturase.

Additionally, the present invention includes a method (i.e., "first" method) for producing a polyunsaturated fatty

acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:1 (Figure 12); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of the human  $\Delta 5$ -desaturase enzyme; and d) exposing the expressed human  $\Delta 5$ -desaturase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, DGLA or 20:4n-3 and the product polyunsaturated fatty acid may be, for example, AA or EPA, respectively. This method may further comprise the step of exposing the product polyunsaturated fatty acid to an elongase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid (i.e., "second" method). In this method containing the additional step (i.e., "second" method), the product polyunsaturated fatty acid may be, for example, AA or EPA, and the "another" polyunsaturated fatty acid may be adrenic acid or (n-3)-docosapentaenoic acid, respectively. The method containing the additional step may further comprise a step of exposing the another polyunsaturated fatty acid to an additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid (i.e., "third" method). The final polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or docosahexaenoic (DHA) acid.

The present invention also encompasses a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the "first" method, another polyunsaturated fatty acid produced according to the "second" method, and the final polyunsaturated fatty acid

produced according to the "third" method. The product polyunsaturated fatty acid may be, for example, AA or EPA. The another polyunsaturated fatty acid may be, for example, adrenic acid or (n-3)-docosapentaenoic acid. The final  
5 polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or DHA. This nutritional composition, may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or to an animal. It may be administered enterally or parenterally. The  
10 nutritional composition may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and protein hydrolysates. Additionally,  
15 the composition may further comprise at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and  
20 iron.

Furthermore, the present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the  
25 "first" method, the another polyunsaturated fatty acid produced according to the "second" method, and the final polyunsaturated fatty acid produced according to the "third" method and 2) a pharmaceutically acceptable carrier. Again, the pharmaceutical composition may be administered to a human or to an animal.  
30 The composition may further comprise an element selected from the group consisting of a vitamin, a mineral, a carbohydrate, an

amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

Additionally, the present invention includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the first method, the another polyunsaturated fatty acid produced according to the second method and the final polyunsaturated fatty acid produced according to the third method. The product polyunsaturated fatty acid may be, for example, AA or EPA. The another polyunsaturated fatty acid may be, for example, adrenic acid or (n-3)-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or DHA.

Moreover, the present invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the first method, the another polyunsaturated fatty acid produced according to the second method, and the final polyunsaturated fatty acid produced according to the third method.

Additionally, the present invention encompasses a method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the nutritional composition of above in an amount sufficient to effect prevention or treatment.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 outlines the sections of the *M. alpina*  $\Delta 5$ - and  $\Delta 6$ -desaturases, the clone ID's from the LifeSeq database to which those sections had homology, and the keyword associated with the clone ID's.

Figure 2 represents the contig 2692004.

Figure 3 represents the contig 2153526.

Figure 4 represents the contig 3506132.

Figure 5 represents the contig 3854933.

5 Figure 6 represents the contig 2511785.

Figure 7 represents the contig 2535 generated based on contig 2511785 of Figure 6 and contig 3506132 of Figure 4.

Figure 8 represents the contig 253538a generated based on contig 2535 of Figure 7 and contig 3854933 of Figure 5.

10 Figure 9 represents the amino acid sequence identity between the *M. alpina*  $\Delta 5$ -desaturase (Ma29) and the contig 253538a.

Figure 10 represents the amino acid sequence identity between the *M. alpina*  $\Delta 6$ -desaturase (Ma524) and the contig  
15 253538a.

Figure 11 represents various fatty acid biosynthesis pathways. The role of the  $\Delta 5$ -desaturase enzyme should be noted.

Figure 12 represents the complete nucleotide sequence of the human  $\Delta 5$ -desaturase gene (human  $\Delta 5$ ).

20 Figure 13 represents the amino acid sequence of the human  $\Delta 5$ -desaturase translated from human  $\Delta 5$  (see Figure 12).

Figure 14 illustrates the sequence identity between the pRAE-7 and pRAE-8 clones.

Figure 15 represents the complete putative human desaturase  
25 gene sequence from clone pRAE-7.

Figure 16 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the *M. alpina*  $\Delta 5$ -desaturase.

Figure 17 illustrates the amino acid sequence identity  
30 between the putative human desaturase gene in pRAE-7 and the *M. alpina*  $\Delta 6$ -desaturase.

Figure 18 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the contig 2535.

Figure 19 illustrates the amino acid sequence identity  
5 between the putative human desaturase gene in pRAE-7 and the contig 38.

Figure 20 illustrates the amino acid sequence identity between the N-terminus of clone A-1, a representative of Group 1, and the N-terminus of cytochrome b5 gene.

10 Figure 21 illustrates the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-1 and a portion of the GenBank sequence ac004228.

Figure 22 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone 3-5 of  
15 Group 2 and a portion of the GenBank sequence ac004228. Clone 3-5 has an ATG within a *NcoI* site, but translates four stops between the ATG and the *BamHI* site.

Figure 23 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-10 of  
20 Group 3 and a portion of the GenBank sequence ac004228. Clone A-10 has an ATG 135 bp upstream of the *BamHI* site, giving an open reading frame of 1267 bp.

Figure 24 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-16 of  
25 Group 4 and a portion of the GenBank sequence ac004228. Clone A-16 does not have an ATG; however, there is an ATG (underlined) upstream of where the sequence aligns with ac004228.

Figure 25 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-19 of  
30 Group 5 and a portion of the GenBank sequence ac004228. Clone A-19 does not have an ATG; however, this clone matches the ac004228 sequence even upstream of the *BamHI* site.



Figure 26 represents the partial nucleotide sequence of the GenBank sequence ac004228 and the representative clones from the five Groups.

Figure 27 represents the nucleotide sequence identity  
5 between the human  $\Delta 5$ -desaturase and contig 3381584.

Figure 28 represents the nucleotide sequence identity between the human  $\Delta 5$ -desaturase and contig 2153526.

Figure 29 represents the amino acid sequence identity between the human  $\Delta 5$ -desaturase and contig 253538a.

10 Figure 30 represents the amino acid sequence identity between the human  $\Delta 5$ -desaturase and contig 38.

Figure 31 represents the amino acid sequence identity between the *M. alpina*  $\Delta 6$ -desaturase (Ma524) and the human the  $\Delta 5$ -desaturase.

15 Figure 32 represents the amino acid sequence identity between the *M. alpina*  $\Delta 5$ -desaturase (Ma29) and the human  $\Delta 5$ -desaturase.

Figure 33 illustrates the human  $\Delta 5$ -desaturase activity of the gene in clone pRAE-28-5, compared to that in pRAE-26-1,  
20 pRAE-33, and pRAE-35, when expressed in baker's yeast.

Figure 34 illustrates the substrate specificity of the human  $\Delta 5$ -desaturase gene in clone pRAE-28-5, converting DGLA(20:3n-6) to AA(20:4n-6), when the gene is expressed in baker's yeast.

#### 25 DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to the nucleotide and amino acid sequence of the  $\Delta 5$ -desaturase gene derived from humans. Furthermore, the subject invention also includes uses of the gene and of the enzyme encoded by this gene. For example, the  
30 gene and corresponding enzyme may be used in the production of polyunsaturated fatty acids such as, for instance, arachidonic

acid, eicosapentaenoic acid, and/or adrenic acid which may be added to pharmaceutical compositions, nutritional compositions and to other valuable products.

5    The Human  $\Delta$ 5-Desaturase Gene and Enzyme Encoded Thereby

As noted above, the enzyme encoded by the human  $\Delta$ 5-desaturase gene is essential in the production of highly unsaturated polyunsaturated fatty acids having a length greater than 20 carbons. The nucleotide sequence of the isolated human  
10    $\Delta$ 5-desaturase gene is shown in Figure 2, and the amino acid sequence of the corresponding purified protein is shown in Figure 3.

As an example, the isolated human  $\Delta$ 5-desaturase gene of the present invention converts DGLA to AA or converts 20:4n-3 to  
15   EPA. Thus, neither AA nor EPA, for example, can be synthesized without the  $\Delta$ 5-desaturase gene (e.g., human or *M. alpina*) and enzyme encoded thereby.

It should be noted that the present invention also encompasses nucleotide sequences (and the corresponding encoded  
20   proteins) having sequences corresponding to or complementary to at least about 50%, preferably at least about 60%, and more preferably at least about 70% of the nucleotides in sequence to SEQ ID NO:1 (i.e., the nucleotide sequence of the human  $\Delta$ 5-desaturase gene described herein (see Figure 12)). Such  
25   sequences may be derived from non-human sources (e.g., *C. elegans* or mouse). Furthermore, the present invention also encompasses fragments and derivatives of the nucleotide sequence of the present invention (i.e., SEQ ID NO:1), as well as of the sequences derived from non-human sources, and having the above-  
30   described complementarity or correspondence. Functional equivalents of the above-sequences (i.e., sequences having human

Δ5-desaturase activity) are also encompassed by the present invention. The invention also includes a purified polypeptide which desaturates polyunsaturated fatty acids at the carbon 5 position and has at least about 50% amino acid similarity to the amino acid sequence of the above-noted proteins which are, in turn, encoded by the above-described nucleotide sequences.

The present invention also encompasses an isolated nucleotide sequence which encodes PUFA desaturase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence corresponding to or complementary to the nucleotide sequence represented by SEQ ID NO:1 and shown in Figure 12. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. "Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation. Such variables are well known in the art. More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value of  $T_m$  for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating  $T_m$  have been derived (see Sambrook et al., *supra*). For hybridization with shorter nucleic acids, the position of mismatches becomes more

important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*).

#### Production of the Human $\Delta 5$ -Desaturase Enzyme

5

Once the gene encoding the human  $\Delta 5$ -desaturase enzyme has been isolated, it may then be introduced into either a prokaryotic or eukaryotic host cell through the use of a vector or construct.

10

The vector, for example, a bacteriophage, cosmid or plasmid, may comprise the nucleotide sequence encoding the human  $\Delta 5$ -desaturase enzyme as well as any promoter which is functional in the host cell and is able to elicit expression of the human  $\Delta 5$ -desaturase encoded by the nucleotide sequence. The promoter is in operable association with or operably linked to the nucleotide sequence. (A promoter is said to be "operably linked" with a coding sequence if the promoter affects transcription or expression of the coding sequence.) Suitable promoters include, for example, those from genes encoding alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglucosomerase, phosphoglycerate kinase, acid phosphatase, T7, TPI, lactase, metallothionein, cytomegalovirus immediate early, whey acidic protein, glucoamylase, and promoters activated in the presence of galactose, for example, GAL1 and GAL10. Additionally, nucleotide sequences which encode other proteins, oligosaccharides, lipids, etc. may also be included within the vector as well as other regulatory sequences such as a polyadenylation signal (e.g., the poly-A signal of SV-40T-antigen, ovalalbumin or bovine growth hormone). The choice of sequences present in the construct is dependent upon the desired expression products as well as the nature of the host cell.

30

As noted above, once the vector has been constructed, it may then be introduced into the host cell of choice by methods known to those of ordinary skill in the art including, for example, transfection, transformation and electroporation (see 5 Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)). The host cell is then cultured under suitable conditions permitting expression of the desired PUFA which is then recovered and purified.

10 Examples of suitable prokaryotic host cells include, for example, bacteria such as Escherichia coli, Bacillus subtilis as well as cyanobacteria such as Spirulina spp. (i.e., blue-green algae). Examples of suitable eukaryotic host cells include, for example, mammalian cells, plant cells, yeast cells such as 15 Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Lipomyces starkey, Candida spp. such as Yarrowia (Candida) lipolytica, Kluyveromyces spp., Pichia spp., Trichoderma spp. or Hansenula spp., or fungal cells such as filamentous fungal cells, for example, Aspergillus, Neurospora and Penicillium. 20 Preferably, Saccharomyces cerevisiae (baker's yeast) cells are utilized.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals 25 functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such 30 inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that

autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the  
5 marker. When stable expression results from integration, the site of the construct's integration can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs  
10 are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

A transgenic mammal may also be used in order to express the enzyme of interest (i.e., the human  $\Delta 5$ -desaturase), and  
15 ultimately the PUFA(s) of interest. More specifically, once the above-described construct is created, it may be inserted into the pronucleus of an embryo. The embryo may then be implanted into a recipient female. Alternatively, a nuclear transfer method could also be utilized (Schnieke et al., Science  
20 278:2130-2133 (1997)). Gestation and birth are then permitted (see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No. 5,700,671). Milk, tissue or other fluid samples from the offspring should then contain altered levels of PUFAs, as compared to the levels normally found in the non-transgenic  
25 animal. Subsequent generations may be monitored for production of the altered or enhanced levels of PUFAs and thus incorporation of the gene encoding the human  $\Delta 5$ -desaturase enzyme into their genomes. The mammal utilized as the host may be selected from the group consisting of, for example, a mouse,  
30 a rat, a rabbit, a pig, a goat, a sheep, a horse and a cow. However, any mammal may be used provided it has the ability to incorporate DNA encoding the enzyme of interest into its genome.

For expression of a human  $\Delta 5$ -desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the desaturase polypeptide. Transcriptional and translational  
5 initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue  
10 and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is harvested early, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location with the plant by utilizing specific regulatory sequence such as those of U.S. Patent Nos. 5,463,174,  
15 4,943,674, 5,106,739, 5,175,095, 5,420,034, 5,188,958, and 5,589,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. Expression of a human  $\Delta 5$ -  
20 desaturase gene, or antisense human  $\Delta 5$ -desaturase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The human  $\Delta 5$ -desaturase polypeptide coding region may be expressed either by itself or with other genes, in order to produce tissues and/or  
25 plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region may be derived from the 3' region of the gene from which the initiation region was obtained or from a  
30 different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination

region usually is selected as a matter of convenience rather than because of any particular property.

As noted above, a plant (e.g., Glycine max (soybean) or Brassica napus (canola)) or plant tissue may also be utilized as  
5 a host or host cell, respectively, for expression of the human  $\Delta 5$ -desaturase enzyme which may, in turn, be utilized in the production of polyunsaturated fatty acids. More specifically, desired PUFAs can be expressed in seed. Methods of isolating seed oils are known in the art. Thus, in addition to providing  
10 a source for PUFAs, seed oil components may be manipulated through the expression of the human  $\Delta 5$ -desaturase gene, as well as perhaps other desaturase genes and elongase genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and  
15 cosmetics. Once again, a vector which comprises a DNA sequence encoding the human  $\Delta 5$ -desaturase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and under conditions sufficient for expression of the human  $\Delta 5$ -desaturase gene. The vector may also comprise one or more genes  
20 that encode other enzymes, for example,  $\Delta 4$ -desaturase, elongase,  $\Delta 6$ -desaturase,  $\Delta 12$ -desaturase,  $\Delta 15$ -desaturase,  $\Delta 17$ -desaturase, and/or  $\Delta 19$ -desaturase. The plant tissue or plant may produce the relevant substrate (e.g., DGLA, GLA, EPA, 20:4n-3, etc.) upon which the enzymes act or a vector encoding enzymes which  
25 produce such substrates may be introduced into the plant tissue, plant cell or plant. In addition, substrate may be sprayed on plant tissues expressing the appropriate enzymes. Using these various techniques, one may produce PUFAs (e.g., n-6 unsaturated fatty acids such as AA, or n-3 fatty acids such as EPA or DHA)  
30 by use of a plant cell, plant tissue or plant. It should also be noted that the invention also encompasses a transgenic plant



comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in, for example, the seeds of the transgenic plant.

5           The substrates which may be produced by the host cell either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector which is subsequently introduced into the host cell, are shown in Figure 11.

10           In view of the above, the present invention encompasses a method of producing the human  $\Delta 5$ -desaturase enzyme comprising the steps of: 1) isolating the nucleotide sequence of the gene encoding human  $\Delta 5$ -desaturase enzyme; 2) constructing a vector comprising said nucleotide sequence; and 3) introducing said  
15   vector into a host cell under time and conditions sufficient for the production of the desaturase enzyme.

          The present invention also encompasses a method of producing polyunsaturated fatty acids comprising exposing an acid to the human  $\Delta 5$ -desaturase enzyme such that the desaturase  
20   converts the acid to a polyunsaturated fatty acid. For example, when 20:3n-6 is exposed to human  $\Delta 5$ -desaturase enzyme, it is converted to AA. AA may then be exposed to elongase which elongates the AA to adrenic acid (i.e., 22:4n-6).

          Alternatively, human  $\Delta 5$ -desaturase may be utilized to convert  
25   20:4n-3 to 20:5n-3 which may be exposed to elongase and converted to (n-3)-docosapentaenoic acid. The (n-3)-docosapentaenoic acid may then be converted to DHA by use of  $\Delta 4$ -desaturase. Thus, human  $\Delta 5$ -desaturase may be used in the production of polyunsaturated fatty acids which may be used, in  
30   turn, for particular beneficial purposes.

Uses of the Human  $\Delta 5$ -Desaturase Gene and Enzyme Encoded Thereby

As noted above, the isolated human  $\Delta 5$ -desaturase gene and the desaturase enzyme encoded thereby have many uses. For example, the gene and corresponding enzyme may be used indirectly or directly in the production of polyunsaturated fatty acids, for example, AA, adrenic acid or EPA. ("Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter of which is utilized in a composition (e.g., the conversion of DGLA to AA). "Indirectly" is meant to encompass the situation where an acid is converted to another acid (i.e., a pathway intermediate) by the desaturase (e.g., DGLA to AA) and then the latter acid is converted to another acid by use of a non-desaturase enzyme (e.g., AA to adrenic acid by elongase or by use of another desaturase enzyme (e.g., AA to EPA by  $\Delta 17$ -desaturase.)). These polyunsaturated fatty acids (i.e., those produced either directly or indirectly by activity of the desaturase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses are described, in detail, below.

Nutritional Compositions

The present invention includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention comprises at least one oil or acid produced directly or indirectly by use of the human  $\Delta 5$ -desaturase gene, in accordance with the present invention, and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may be added to the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present invention will be of semi-purified or purified origin. By semi-purified or purified is meant a material which

has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, dietary substitutes, and rehydration compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulas, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

The nutritional composition of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

In a preferred embodiment of the present invention, the nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product. This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored

in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio). An oil or acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form, the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and, more preferably, less than 660 mOsm.

The nutritional formula may include macronutrients, vitamins, and minerals, as noted above, in addition to the PUFAs produced in accordance with the present invention. The presence of these additional components helps the individual ingest the minimum daily requirements of these elements. In addition to the provision of PUFAs, it may also be desirable to add zinc, copper, folic acid and antioxidants to the composition. It is believed that these substance boost a stressed immune system and will therefore provide further benefits to the individual receiving the composition. A pharmaceutical composition may also be supplemented with these elements.

In a more preferred embodiment, the nutritional composition comprises, in addition to antioxidants and at least one PUFA, a source of carbohydrate wherein at least 5 weight percent of the carbohydrate is indigestible oligosaccharide. In a more preferred embodiment, the nutritional composition additionally comprises protein, taurine, and carnitine.

As noted above, the PUFAs produced in accordance with the present invention, or derivatives thereof, may be added to a dietary substitute or supplement, particularly an infant formula, for patients undergoing intravenous feeding or for preventing or treating malnutrition or other conditions or disease states. As background, it should be noted that human breast milk has a fatty acid profile comprising from about 0.15% to about 0.36% as DHA, from about 0.03% to about 0.13% as EPA, from about 0.30% to about 0.88% as AA, from about 0.22% to about 0.67% as DGLA, and from about 0.27% to about 1.04% as GLA. Thus, fatty acids such as AA, EPA and/or docosahexaenoic acid (DHA), produced in accordance with the present invention, can be used to alter, for example, the composition of infant formulas in order to better replicate the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-human mammal's milk. In particular, a composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of AA, DGLA and GLA. More preferably, the oil will comprise from about 0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about 0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention. The preferred composition has about 1 to about 25 weight percent of the total PUFA composition as GLA (U.S. Patent No. 5,196,198). Other vitamins, particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included. When desired, a preservative such as alpha-tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast

milk supplement or substitute, a composition which comprises one or more of AA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to AA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA to AA can be used to produce an AA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% TO 80% can be used to produce an AA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of human  $\Delta 5$ -desaturase expression, as well as the expression of other desaturases and elongases, can be used to modulate PUFA levels and ratios. The PUFAs/acids produced in accordance with the present invention (e.g., AA and EPA) may then be combined with other PUFAs/acids (e.g., GLA) in the desired concentrations and ratios.

Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

#### Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the acids and/or resulting oils produced using the human  $\Delta 5$ -desaturase gene, in accordance with the methods described herein. More specifically, such a

pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with



antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented above.

For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as Intralipids™. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical compositions of the present invention include, for example, enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants in order to form a spray or inhalant.

The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.

The present invention also includes the treatment of  
5 various disorders by use of the pharmaceutical and/or  
nutritional compositions described herein. In particular, the  
compositions of the present invention may be used to treat  
restenosis after angioplasty. Furthermore, symptoms of  
inflammation, rheumatoid arthritis, asthma and psoriasis may  
10 also be treated with the compositions of the invention.  
Evidence also indicates that PUFAs may be involved in calcium  
metabolism; thus, the compositions of the present invention may,  
perhaps, be utilized in the treatment or prevention of  
osteoporosis and of kidney or urinary tract stones.

15 Additionally, the compositions of the present invention may  
also be used in the treatment of cancer. Malignant cells have  
been shown to have altered fatty acid compositions. Addition of  
fatty acids has been shown to slow their growth, cause cell  
death and increase their susceptibility to chemotherapeutic  
20 agents. Moreover, the compositions of the present invention may  
also be useful for treating cachexia associated with cancer.

The compositions of the present invention may also be used  
to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et  
al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered  
25 fatty acid metabolism and composition have been demonstrated in  
diabetic animals.

Furthermore, the compositions of the present invention,  
comprising PUFAs produced either directly or indirectly through  
the use of the human  $\Delta 5$ -desaturase enzyme, may also be used in  
30 the treatment of eczema, in the reduction of blood pressure, and  
in the improvement of mathematics examination scores.  
Additionally, the compositions of the present invention may be

used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976),  
5 reduction or prevention of gastrointestinal bleeding and other side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after  
10 viral infections (see U.S. Patent No. 5,116,871).

Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and inflammatory skin disorders, as well as for maintenance of general health.

15 Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

## 20 Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals (i.e., domestic or non-domestic), as well as humans, as  
25 animals experience many of the same needs and conditions as humans. For example, the oil or acids of the present invention may be utilized in animal feed supplements, animal feed substitutes, animal vitamins or in animal topical ointments.

The present invention may be illustrated by the use of the  
30 following non-limiting examples:

EXAMPLE IHUMAN DESATURASE GENE SEQUENCES

As described in International Application PCT/US98/07422 (herein incorporated in its entirety by reference), the putative  
5 human desaturase gene sequences involved in long chain polyunsaturated fatty acid biosynthesis were isolated based on homology between the human cDNA sequences and *Mortierella alpina* desaturase gene sequences. The three conserved "histidine boxes" known to be conserved among membrane-bound desaturases  
10 were found. As with other membrane-bound desaturases, the final HXXHH histidine box motif was found to be QXXHH. The amino acid sequence of the putative human desaturases exhibited homology to *M. alpina*  $\Delta 5$ -,  $\Delta 6$ -,  $\Delta 9$ -, and  $\Delta 12$ -desaturases.

The *M. alpina*  $\Delta 5$ -desaturase and  $\Delta 6$ -desaturase cDNA  
15 sequences were used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, CA. The  $\Delta 5$ -desaturase sequence was divided into fragments: 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-446. The  $\Delta 6$  desaturase sequence was divided into three fragments: 1) amino  
20 acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-457. These polypeptide fragments were searched against the database using the "tblastn" algorithm. This algorithm compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands).

25 The polypeptide fragments 2 and 3 of *M. alpina*  $\Delta 5$ - and  $\Delta 6$ -desaturases have homologies with the CloneID sequences as outlined in Figure 1. The CloneID represents an individual sequence from the Incyte LifeSeq database. After the "tblastn" results had been reviewed, Clone Information was searched with  
30 the default settings of Stringency of  $\geq 50$ , and Productscore  $\leq 100$  for different CloneID numbers. The Clone Information

Results displayed the information including the ClusterID, CloneID, Library, HitID, and Hit Description. When selected, the ClusterID number displayed the clone information of all the clones that belong in that ClusterID. The Assemble command  
5 assembled all of the CloneID which comprise the ClusterID. The following default setting were used for GCG (Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, WI) Assembly:

Word Size: 7; Minimum Overlap: 14; Stringency: 0.8; Minimum  
10 Identity: 14; Maximum Gap: 10; Gap Weight: 8; and Length Weight: 2.

GCG Assembly Results displayed the contigs generated on the basis of sequence information within the CloneID. A contig is an alignment of DNA sequences based on areas of homology among  
15 these sequences. A new sequence (consensus sequence) was generated based on the aligned DNA sequence within a contig. The contig. containing the CloneID was identified, and the ambiguous sites of the consensus sequence were edited based on the alignment of the CloneIDs (see Figures 2-6) to generate the  
20 best possible sequence. The procedure was repeated for all six CloneID listed in Figure 1. This produced five unique contigs. The edited consensus sequences of the 5 contigs were imported into the Sequencher software program (Gene Codes Corporation, Ann Arbor, Michigan). These consensus sequences were assembled.  
25 The contig 2511785 overlaps with contig 3506132, and this new contig was called 2535 (Figure 7). The contigs from the Sequencher program were copied into the Sequence Analysis software package of GCG.

Each contig was translated in all six reading frames into  
30 protein sequences. The *M. alpina*  $\Delta 5$ -desaturase (Ma29) and  $\Delta 6$ -desaturase (Ma524) sequences were compared with each of the translated contigs using the FastA search (a Pearson and Lipman

search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein)). Homology among these sequences suggest the open reading frames of each contig as underlined in Figures 3, 5, and 7. The homology among the *M. alpina*  $\Delta 5$ - and  $\Delta 6$ -desaturase sequences to contigs 2535 and 3854933 were utilized to create the final contig called 253538a (see Figure 8). Figure 9 is the FastA match of the translated sequences of the final contig 253538a and Ma29, and Figure 10 is the FastA match of the translated sequences of the final contig 253538a and Ma524.

Although the open reading frame was generated by merging the two contigs, the contig 2535 shows that there is a unique sequence in the beginning of this contig which does not match with the contig 3854933. Therefore, it is possible that these contigs were generated from independent desaturase-like human genes.

The contig 253538a contains an open reading frame encoding 432 amino acid (Figure 8, underlined). It starts with Gln (CAG) and ends with the stop codon (TGA) (both in bold). The contig 253538a aligns with both *M. alpina*  $\Delta 5$ - and  $\Delta 6$ -desaturase sequences, suggesting that it could be either of the desaturases, as well as other known desaturases which share homology with each other. The individual contigs listed in Figure 1, as well as the intermediate contig 2535 and the final contig 253538a can be utilized to isolate the complete genes for human desaturases.

#### Determination of Human $\Delta 5$ -Desaturase Gene Sequence

Primers RO384 and RO388 were designed based on the 5' and 3' sequences, respectively, of contig 2535. The human monocyte cDNA library (Clontech, Palo Alto, CA) was amplified with the vector primer RO329 (5'- CAG ACC AAC TGG TAA TGG TAG - 3') and

RO384 (5' - TCA GGC CCA AGC TGG ATG GCT GCA ACA TG - 3'), and also with the vector primer RO328 (5' - CTC CTG GAG CCC GTC AGT ATC - 3') and RO388 (5' - ATG GTG GGG AAG AGG TGG TGC TCA ATC TG - 3'). Polymerase Chain Reaction (PCR) was carried out in a 100  $\mu$ l volume containing: 1  $\mu$ l of human monocyte cDNA library, 10 pM each primer, 10  $\mu$ l of 10X buffer and 1.0 U of Taq Polymerase. Thermocycler conditions in Perkin Elmer 9600 were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins. and 72 °C for 3 mins. PCR was followed by an additional extension at 72 °C for 7 minutes.

The PCR amplified mixture was run on a gel, and the amplified fragments were gel purified. The isolated fragment from PCR amplification with RO329 and RO384 was approximately 900 bp, and that from PCR amplification with RO328 and RO388 was approximately 650 bp. These isolated fragments were filled-in using T4 DNA polymerase, and the filled-in fragments were cloned into the PCR-Blunt vector (Invitrogen Corp., Carlsbad, CA). The clone of RO329/RO384 amplified fragment was designated as pRAE-7, and the clone of RO328/RO388 amplified fragment was designated as pRAE-8. Both ends of the clones were sequenced using ABI 373 DNA Sequencer (Applied Biosystems, Foster City, CA) and assembled using the Sequencher program (a sequence analysis program, Gene Codes Corporation, Ann Arbor, MI). This assembly of the sequences revealed that the two clones contained different sizes of the same gene (Figure 14). The complete sequence of the pRAE-7 gene was compiled (Figure 15) and searched against the known sequences in the public database.

The FastA algorithm is a Pearson and Lipman search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein). The pRAE-7 gene sequence was translated in six reading frames, and using this method, the Swissprot database (Genetics Computer Group (GCG)

(Madison, WI) was searched. The gene in pRAE-7 was identified as a putative human desaturase based on its homology to known desaturases. The Swissprot database search produced matches against the omega-3 fatty acid desaturase from mung bean (23.4% identity in 303 AA overlap), linoleoyl-CoA desaturase from *Synechocystis* sp. (24.3% identity in 280 AA overlap), omega-6 fatty acid desaturase from soybean (19.7% identity in 284 AA overlap), and acyl-CoA desaturase 1 from *Saccharomyces cerevisiae* (21.6% identity in 134 AA overlap). The FastA search against the *M. alpina* desaturases produced matches against the  $\Delta 6$ - (31.9% identity in 285 AA overlap), the  $\Delta 5$ - (28.4% identity in 292 AA overlap), and the  $\Delta 12$ - (23.0% identity in 274 AA overlap) desaturases. The matched sequence alignment of the putative human desaturase gene in pRAE-7 against *M. alpina*  $\Delta 5$ -desaturase (Ma29), *M. alpina*  $\Delta 6$ -desaturase (Ma524) as well as to the contigs 2535 and 38 are displayed in Figures 16, 17, 18, and 19 respectively.

The contigs 2535, 38, and 253538a were generated based on assemblies of various sequences as well as their homologies against the known desaturases. However, upon examining Figures 18 and 19, it can be concluded that the contigs are merely indications as to what the sequences of the human desaturases might possibly be.

The 5' end of the gene, the ATG (Methionine), is necessary for expressing the human desaturase in yeast. Figures 16 and 17 show that pRAE-7 is probably just the last 2/3 of a desaturase gene. Several of the omega-3 and omega-6 fatty acid desaturases, as well as the linoleoyl-CoA desaturase mentioned above, are smaller than the *M. alpina*  $\Delta 5$ - and  $\Delta 6$ -desaturases, ranging in sizes of 359-380 amino acids. It was concluded from all of the sequences evaluated thus far that the isolated gene



probably needed anywhere from 180-480bp (60-160 amino acids) of additional 5' sequence for expressing a complete enzyme.

In order to extend the 5' sequence of the human desaturase gene, the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA) was used to screen the human liver marathon ready cDNA (Clontech). The rapid amplification of cDNA ends (RACE) reactions are efficient for both 5' and 3' long-distance PCR. Following the 5' RACE protocol outlined in the kit, the primers RO430 (5' - GTG GCT GTT GTT ATT GGT GAA GAT AGG CAT C - 3') (designed based on the pRAE-7 gene 3' sequence, downstream of the TAA (stop)) and the marathon adaptor primer (AP1) from the kit, were used to generate three PCR amplified products, which were designated A, B, and C. The fragment sizes were approximately 1.5 Kb, 1.4 Kb, 1.2 Kb, respectively. The fragments were filled-in with T4 DNA polymerase, and cloned into the PCR-blunt vector. A total of twenty-two clones were generated and sequenced. Using the FastA algorithm, the sequences were searched against the GenEMBL database of GCG.

Many of the sequences had a great homology to the human DNA sequence with the GenBank accession number of AC004228. This DNA sequence is described as: Sequencing in Progress, *Homo sapiens* Chromosome 11q12pac pDJ519o3; HTGS phase 1,18 unordered pieces. The 18 contigs were recorded in an arbitrary fashion. Using this sequence information and the information from the assembled sequences of the clones, the clones were categorized into five groups.

All of the clones have the same sequence downstream of the *Bam*HI site (see Figure 12, underlined). But each group represents a different 5' sequence, with a total of 10 clones being too short to be the full length gene. Group 1, represented by clone A-1, is comprised of 5 clones which have homology to cytochrome b5 gene (Figure 20). A translational

start codon, ATG, is not present in clone A-1; however, as can be seen in Figure 21, there is an ATG (underlined) present in the ac004228 sequence 17 bp upstream of the strong area of homology between A-1 and ac004228. Starting from the strong area of homology, A-1 has an open reading frame of 1318 bp. However, starting from the ATG, the open reading frame is 1335 bp. Group 2, represented by clone 3-5, is comprised of 3 clones which have an ATG within an *NcoI* site, but four translational stop codons between the ATG and the *BamHI* site (Figure 22, the *NcoI*, *BamHI* sites are in bold, and the four termination codons are underlined). Group 3 is comprised of one clone, A-10, which has an ATG 135 bp upstream of the *BamHI* site, giving an open reading frame of 1267 bp (Figure 23). Group 4 is comprised of 2 clones, represented by clone A-16, which does not have an ATG; however, upstream of where the sequence aligns with ac004228, there is an ATG (Figure 24, underlined). The open reading frame of this group is 1347 bp. Group 5 is comprised of one clone which does not have an ATG. However, this clone matches the ac004228 sequence even upstream of the *BamHI* site (Figure 25).

As illustrated in Figure 26, many of the clones from the five groups are represented in order with the ac004228 sequence. There appeared to be a high level of splicing, with the sequence downstream of the *BamHI* site (in bold) acting as the common anchor for the various 5' exons. All of the potential start sites are also in bold, and the sequences found within the clones have been underlined.

The A-1 sequence was used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, CA, to see if its latest version would also have sequences with homology to our desaturase gene sequence. Two contigs were generated in this search, contig 3381584 and contig 2153526. The human desaturase gene sequence was initially compiled based on sequences from

Group I clones and ac004228. However, Figure 12 represents the actual DNA sequence of the isolated gene. The Incyte contigs were used to confirm this sequence (see Figures 27 and 28). The human desaturase translated sequence, consisting of 445 amino acids (Figure 13), was also matched with the original contigs 253538a and 38. These alignments are shown in Figures 29 and 30, respectively.

The FastA search of the human desaturase gene against the Swissprot database produced matches against the omega-3 fatty acid desaturase from mung bean (22.4% identity in 381 AA overlap), linoleoyl-CoA desaturase from *Synechocystis* Sp. (24.5% identity in 335 AA overlap), omega-6 fatty acid desaturase from soybean (20.3% identity in 290 AA overlap), and acyl-CoA desaturase 1 from *Saccharomyces cerevisiae* (21.4% identity in 168 AA overlap). The FastA search against *M. alpina* desaturases produced matches against the  $\Delta 6$ - (30.5% identity in 455 AA overlap),  $\Delta 5$ - (27.5% identity in 455 AA overlap), and  $\Delta 12$ - desaturases (22.5% identity in 382 AA overlap). The FastA match of the human desaturase translated sequence against the ma524 (*M. alpina*  $\Delta 6$ -desaturase) and ma29 (*M. alpina*  $\Delta 5$ -desaturase) sequences are shown in Figures 31 and 32, respectively.

## EXAMPLE II

### CONSTRUCTION OF CLONES

New clones were generated based on clones from three of the Groups mentioned above, clones A-1, A-10, and A-16. Two primers which were modified with 5' phosphate, R0526 (5'-CAT GGC CCC CGA CCC GGT GG-3') and R0527 (5'-GCG GCC ACC GGG TCG GGG GC-3'), were annealed together to form an adaptor. This adaptor which has *Nco*I and *Bsa*I overhangs, were ligated with the A-1 clone, which had been cut with *Bsa*I/*Hind*III and gel purified, for 15 min at room temperature. The pYX242(*Nco*I/*Hind*III) vector

(Novagen, Madison, WI) was added to this ligation mixture and allowed to incubate at room temperature for an additional 45 min. This produced a clone designated as pRAE-28-5. (Plasmid pRAE-28-5 was deposited with the American Type Culture  
5 Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 on December 21, 1998, under the terms of the Budapest Treaty, and was accorded ATCC number \_\_\_\_.)

The A-10 clone was PCR amplified with R0512 (5'-GAT TGG GTG CCA TGG GGA TGC GGG ATG AAA AGG C-3') and R05 (5'-GAA ACA GCT  
10 ATG ACC ATG-3'), the amplified product was cut with *NcoI* and *HindIII* and gel purified, and the purified fragment was cloned into pYX242 (*NcoI/HindIII*). This new clone was designated as pRAE-26-1.

The A-10 clone was also PCR amplified with R0580 (5' - TCC  
15 TGC GAA TTC ACC ATG AAA AGG CGG GAG AGA G - 3') and R05, the amplified product was cut with *NcoI* and *HindIII* and gel purified, and the purified fragment was cloned into pYX242 (*NcoI/HindIII*). This new clone was designated as pRAE-33.

Two primers which were modified with 5' phosphate, R0578  
20 (5' - CAT GGC TAG GAG AGG CAG CGC AGC CGC GTC TGG AC - 3') and R0579 (5' - CTA GGT CCA GAC GCG GCT GCG CTG CCT CTC CTA GC - 3'), were annealed together to form an adaptor. This adaptor which has *NcoI* and *BlnI* overhangs, were ligated with the A-16 clone, which had been cut with *BlnI/HindIII* and gel purified,  
25 for 15 min at room temperature. The pYX242(*NcoI/HindIII*) vector was added to this ligation mixture and allowed to incubate at room temperature for an additional 45 min. This produced a clone designated as pRAE-35.

EXAMPLE IIIEXPRESSION OF HUMAN  $\Delta 5$ -DESATURASE

The constructs pRAE-26-1, pRAE-28-5, pRAE-33, and pRAE-35 were transformed into *S. cerevisiae* 334 and screened for desaturase activity. The substrates DGLA (20:3n-6), OA(18:1n-9), AA(20:4n-6), and LA(18:2n-6) were used to determine the activity of the expressed gene from constructs pRAE-26-1 and pRAE-28-5. Only the substrate DGLA was used to determine the activity of the expressed gene from all of the constructs. The negative control strain was *S. cerevisiae* 334 containing the unaltered pYX242 vector. The cultures were grown for 48 hours at 30°C, in selective media (Ausubel et al., Short Protocols in Molecular Biology, Ch. 13, P. 3-5 (1992)), in the presence of a particular substrate. Lipid fractions of each culture were extracted for analysis. The desaturase activity results are provided in Figures 33 and 34.

All of the values in Figure 33 are the average of two separate samples per strain, tested in the same run. The substrate, as well as the fatty acid it was converted to, is shown in bold. The expressed gene in the strain 334 (pRAE-28-5) is a  $\Delta 5$ -desaturase. It converted the substrate DGLA to a higher percent of AA than the control strain 334(pYX242), 0.127% vs. 0.062%, respectively. The percent of AA present in the cultures of strains 334(pRAE-26-1), 334(pRAE-33), and 334(pRAE-35) are comparable to that of the control strain (0.075%, 0.062%, and 0.063%, respectively). Therefore, it can be concluded that the cyt b5 sequence containing gene in the construct pRAE-28-5 expresses an active human  $\Delta 5$ -desaturase; whereas, the other variations of the gene do not.

The activity of the human  $\Delta 5$ -desaturase was further confirmed in the experiment outlined in Figure 34. Included in

this figure are the fatty acid profiles of the strains 334(pRAE-28-5), 334(pRAE-26-1), and the control strain 334(pYX242) when DGLA(20:3n-6), OA(18:1n-9), AA(20:4n-6), or LA(18:2n-6) was used as the substrate, as well as when no substrate was added.

5 Again, the strain 334(pRAE-28-5) expressed an active human  $\Delta 5$ -desaturase, converting DGLA to AA at a higher percent than the control strain, 0.106% vs. 0.065%, respectively. The strain 334(pRAE-26-1) had about the same amount of AA (0.06%) as the control. The conversion of the substrate OA to LA was not  
10 detected, confirming that the strains do not have a  $\Delta 12$ -desaturase activity. The conversion of the substrate AA to eicosapentaenoic acid (EPA, 20:5n-3) was detected, but at a very low level equal to that of the control strain, confirming that the strains do not have a  $\Delta 17$ -desaturase activity. The  
15 conversion of the substrate LA to GLA was detected, but again at a very low level equal to the control strain, confirming that the strains do not have a  $\Delta 6$ -desaturase activity.

The present sequence (Figure 12) differs from the Genbank sequence g3169158 of the LifeSeq database with respect to two  
20 positions. In particular, with respect to the nucleotide sequence of sequence g3169158, position 1082 is an adenosine; however, in the present sequence position 1082 is a thymine (see Figure 12). Furthermore, position 1229 of sequence g3169158 is an adenine whereas in the present sequence position 1229 is a  
25 guanine. In terms of an amino acid sequence comparison, position 361 of the present sequence is a leucine (see Figure 13), and position 361 of sequence g3169158 is a glutamine. Furthermore, position 410 of the present sequence is an arginine, whereas position 410 of sequence g3169158 is a  
30 histidine. Additionally, sequence g3169158 is described, in the database, as a "hypothetical protein" which "exhibits similarity to motifs found in delta 6 desaturase, a hypothetical cytochrome

b5 containing fusion protein." However, as demonstrated in the above example, the protein encoded by the sequence in Figure 12 is a human  $\Delta 5$ -desaturase, not a  $\Delta 6$ -desaturase.

5

#### EXAMPLE IV

##### EXPRESSION OF HUMAN $\Delta 5$ -DESATURASE IN INSECT CELLS

Insect cells were used as another eukaryotic host for expression of the human  $\Delta 5$ -desaturase. The baculovirus expression system involves the use of insect cells to express a gene, in this case, the human  $\Delta 5$ -desaturase, which has been cloned into a baculovirus expression vector. Insect cells are known to have no endogenous PUFA desaturase activities. Therefore, this system is suitable for expression and characterization of the recombinant desaturases.

The fragment containing the human  $\Delta 5$ -desaturase gene (pRAE-28-5, see EXAMPLE II) was PCR amplified using Expand High Fidelity PCR System (Boehringer Mannheim Corp., Indianapolis, IN) and a set of primers containing appropriate restriction sites. The upstream primer designated RO676 (5'- ATA CGT GAA TTC GCC GCC ACC ATG GCC CCC GAC CCG GTG - 3') corresponded to the sense strand of  $\Delta 5$  cDNA and contained an EcoRI site 5' upstream of the ATG. The downstream primer RO677 (5' - TAT CCG CTC GAG TTA TTG GTG AAG ATA GGC ATC TAG - 3') corresponded to the antisense strand at the 3' end of the  $\Delta 5$  cDNA, and included an XhoI site immediately downstream of the translational termination codon. The PRC reaction, in a final volume of 100  $\mu$ l, was carried out as follows: 5 mins denaturation at 94 °C, then 45 seconds at 94 °C, 45 seconds at 55 °C and 2 min at 72 °C for 30 cycles, and 7 mins. extension at 72 °C at the end of the amplification. The human  $\Delta 5$  PCR amplified product was analyzed by agarose-gel electrophoresis, gel purified, digested with

EcoRI and XhoI, and then ligated into pFastBac1 baculovirus donor plasmid (Gibco-BRL, Gaithersburg, MD) which was restricted with the same enzymes. The respective baculovirus clone was designated as pJPBh4 for the human  $\Delta 5$ -desaturase. This  
5 pFastBac1 vector contains an expression cassette which has a polyhedrin promoter, a SV40 polyadenylation signal, and a gentamycin resistance marker.

The initial transformation was done in XL1 blue cells (Invitrogen, Carlsbad, CA). Positive clones were then  
10 transformed into E. coli DH10Bac (Gibco-BRL, Gaithersburg, MD) which contains the baculovirus genome. The positive clones were selected by blue white screening in which white colonies contain the recombinant bacmid. White colonies were then selected for bacmid DNA isolation. DNA was isolated using a Qiagen plasmid  
15 isolation kit (Qiagen, Inc., Valencia, CA), specific for DNA over 135 kb long. The recombinant bacmid DNA was analyzed on a 0.6% agarose gel to confirm the presence of the high molecular weight DNA. PCR analysis, using pUC/M13 primers (forward 5' - TGT AAA ACG ACG GCC AGT - 3' and reverse 5' - GAA ACA GCT ATG ACC  
20 ATG - 3') was also performed to confirm the correct insert size for the desaturase cDNA within the bacmid.

The Sf9 insect cells (Spodoptera frugiperda) were used for the recombinant bacmid DNA transfection. These cells were grown in serum free media (Gibco-BRL, Gaithersburg, MD). Transfection  
25 was carried out according to the CellFECTIN Sf900 protocol (Gibco-BRL, Gaithersburg, MD). The recombinant virus was recovered by collecting the supernatant at 72 hours post-transfection. A plaque assay was performed on the supernatant to determine the titer of recovered recombinant virion  
30 particles. A recombinant viral stock was made for the expression studies. All infections with the recombinant virus were done during the mid-logarithmic growth phase of the Sf9's



and infected at 5 MOI (Multiplicity of Infection). To analyze the activity of the expressed human  $\Delta 5$ -desaturase gene, the Sf9m cells were plated at a concentration of  $1 \times 10^6$  cells/well in a 6-well tissue culture plate and infected with 100  $\mu$ l of the virus stock (approximately 5 MOI). The substrate, dihomogamma-linolenic acid (DGLA, C20:3n-6) was supplemented at the time of infection, at a concentration of 100  $\mu$ M. A mock infected Sf9, as well as cells infected with a recombinant virus containing the GusA reporter gene, were used as negative controls in each experiment. The medium was collected 48 hours post infection and saved. The cells were collected and submitted for lipid analysis.

For fatty acid analysis, cell pellets were vortexed with 6 ml of methanol, followed by the addition of 12 ml of chloroform and tridecanoin (as internal standard). The mixtures were incubated for at least one hour at room temperature or at 4 °C overnight. The chloroform layer was extracted and filtered through a Whatman filter with one gram of anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated at 40 °C under a stream of nitrogen. The extracted lipids were derivatized to fatty acid methyl esters (FAME) for gas chromatography analysis (GC) by adding 2 ml of 0.5 N potassium hydroxide in methanol to a closed tube. The samples were heated at 95 to 100 °C for 30 minutes and cooled to room temperature. Approximately 2 ml of the 14% boron trifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, 2 ml of water and 1 ml of hexane were added to extract the FAME for GC analysis. The percent conversion was calculated by dividing the product produced by the sum of (the product produced and the substrate) and then multiplying by 100.

The fatty acid synthesis in insect cells infected with recombinant virus containing the human  $\Delta 5$  cDNA is summarized in Table 1. The conversion of the added substrate, DGLA (C20:3n-6), to arachidonic acid (AA, 20:4n-6) was monitored. The quantity of arachidonic acid (AA, 20:4n-6) produced by the human  $\Delta 5$ -desaturase was 9.67% of the total fatty acid versus the control which did not produce any AA. This resulted in a 29.6% conversion of DGLA to AA.

These data indicate that the human  $\Delta 5$ -desaturase can be expressed in another eukaryotic host (insect cells) in a biologically active form as demonstrated by the production of AA.

Table 1

<u>Fatty Acid</u>	<u>Human <math>\Delta 5</math></u>	<u>Control</u>
18:1n-9	19.15	19.99
18:3n-6	2.43	5.18
*20:3n-6	22.95	30.00
20:4n-6 (29.6%)	9.67	ND
22:1n-9	0.11	0.25

\* indicates substrate added

ND indicates None Detected

#### Nutritional Compositions

The PUFAs described in the Detailed Description may be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutritional solutions.

#### I. INFANT FORMULATIONS

A. Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cows milk. A feeding for patients  
5 with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein  
10 allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- Low osmolality (240 mOs/kg water) to reduce risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to  
15 enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- 20 -Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar  
25 (sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11 % calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium  
30 phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric

sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

5

B. Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management of  
10 diarrhea in infants and toddlers.

Features:

- First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.
- 15 -Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.
- Nutritionally complete to meet the nutritional needs of the infant.
- Soy protein isolate with added L-methionine meets or exceeds an  
20 infant's requirement for all essential amino acids.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to  
25 enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
- 30 -1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.

-Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve) 86% water, 4.8% corn syrup, 2.5% sugar  
5 (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut  
oil, 0.77% soy fiber, 0.12% calcium citrate, 0.11% calcium  
phosphate tribasic, 0.10% potassium citrate, potassium chloride,  
potassium phosphate monobasic, mono and diglycerides, soy  
lecithin, carrageenan, magnesium chloride, ascorbic acid, L-  
10 methionine, potassium phosphate dibasic, sodium chloride,  
choline chloride, taurine, ferrous sulfate, m-inositol, alpha-  
tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide,  
calcium pantothenate, cupric sulfate, vitamin A palmitate,  
thiamine chloride hydrochloride, riboflavin, pyridoxine  
15 hydrochloride, folic acid, manganese sulfate, potassium iodide,  
phyloquinone, biotin, sodium selenite, vitamin D3 and  
cyanocobalamin.

C. Isomil® SF Sucrose-Free Soy Formula With Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).
- Sucrose free for the patient who cannot tolerate sucrose.
- Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 75% water, 11.8% hydrolyzed cornstarch, 4.1% soy oil, 4.1 % soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch, 0.38% calcium phosphate tribasic, 0.17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, magnesium chloride, ascorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride,

carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

D. Isomil® 20 Soy Formula With Iron Ready To Feed,  
20 Cal/fl oz.:

Usage: When a soy feeding is desired.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar(sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

E. Similac® Infant Formula:

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a

supplement to breastfeeding is needed or as a routine feeding if breastfeeding is not adopted.

Features:

- Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.
- Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.
- Carbohydrate as lactose in proportion similar to that of human milk.
- Low renal solute load to minimize stress on developing organs.
- Powder, Concentrated Liquid and Ready To Feed forms.

Ingredients: (-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, ascorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamide, ferrous sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

F. Similac® NeoCare Premature Infant Formula With Iron:

Usage: For premature infants' special nutritional needs after hospital discharge. Similac NeoCare is a nutritionally complete formula developed to



provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

Features:

- Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).
- Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.
- Higher levels of protein, vitamins and minerals per 100 calories to extend the nutritional support initiated in-hospital.
- More calcium and phosphorus for improved bone mineralization.

Ingredients: -D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

G. Similac Natural Care Low-Iron Human Milk Fortifier  
Ready To Use, 24 Cal/fl oz.:

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

Ingredients: -D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, sodium citrate, ascorbic acid, calcium carbonate, mono and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine, niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous sulfate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D3, sodium selenite and cyanocobalamin.

Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art.

## II. NUTRITIONAL FORMULATIONS

A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is suitable for use in modified diets, including low-

cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet

Ingredients: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

B. ENSURE® BARS:

Usage: ENSURE BARS are complete, balanced nutrition for supplemental use between or with meals. They provide a delicious, nutrient-rich alternative to other snacks. ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor contains gluten.)

Patient Conditions:

- For patients who need extra calories, protein, vitamins and minerals.
- Especially useful for people who do not take in enough calories and nutrients.
- For people who have the ability to chew and swallow
- Not to be used by anyone with a peanut allergy or any type of allergy to nuts.

Ingredients: Honey Graham Crunch -- High-Fructose Corn Syrup, Soy Protein Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice, Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy Polysaccharide, Glycerine, Whey Protein Concentrate, Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that processes nuts.

Vitamins and Minerals: Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese Sulfate, Riboflavin, Beta Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Molybdate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein: Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

Soy protein isolate	74%
Milk proteins	26%

Fat: Honey Graham Crunch - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, oils, and soy lecithin.

Partially hydrogenated cottonseed and soybean oil	76%
Canola oil	8%
High-oleic safflower oil	8%
Corn oil	4%
Soy lecithin	4%

Carbohydrate: Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

High-fructose corn syrup	24%
Brown sugar	21%
Maltodextrin	12%
Honey	11%
Crisp rice	9%
Glycerine	9%
Soy Polysaccharide	7%
Oat bran	7%

C. ENSURE® HIGH PROTEIN:

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions:

- For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

Features:

- Low in saturated fat
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Excellent source of protein, calcium, and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower

Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	85%
Soy protein isolate	15%

Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

High-oleic safflower oil	40%
Canola oil	30%
Soy oil	30%

The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories,

with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

#### Carbohydrate:

ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

#### Vanilla and other nonchocolate flavors:

Sucrose	60%
Maltodextrin	40%

#### Chocolate:

Sucrose	70%
Maltodextrin	30%

#### D. ENSURE® LIGHT

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.



Patient Conditions:

-For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.

-For healthy adults who don't eat right and need extra nutrition.

Features:

-Low in fat and saturated fat

-Contains 3 g of total fat per serving and < 5 mg cholesterol

-Rich, creamy taste

-Excellent source of calcium and other essential vitamins and minerals

-For low-cholesterol diets

-Lactose-free, easily digested

Ingredients:

French Vanilla: -D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride), Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine

Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

**Protein:**

The protein source is calcium caseinate.

Calcium caseinate	100%
-------------------	------

**Fat:**

The fat source is a blend of two oils: high-oleic safflower and canola.

High-oleic safflower oil	70%
Canola oil	30%

The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the, calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

**Carbohydrate:**

ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and

orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Sucrose	51%
Maltodextrin	49%

Chocolate:

Sucrose	47.0%
Corn Syrup	26.5%
Maltodextrin	26.5%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

Caffeine:

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

#### E. ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

Patient Conditions:

- For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume
- For patients who need to gain or maintain healthy weight

Features:

- Rich, creamy taste
- Good source of essential vitamins and minerals

Ingredients:

Vanilla: -D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

The fat source is corn oil.

Corn oil	100%
----------	------

Carbohydrate:

ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, butter pecan, and eggnog), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla, strawberry, butter pecan, and coffee flavors:

Corn Syrup	39%
Maltodextrin	38%
Sucrose	23%

Chocolate and eggnog flavors:

Corn Syrup	36%
Maltodextrin	34%
Sucrose	30%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

Caffeine:

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz.  
Coffee flavor contains a trace amount of caffeine.

## F. ENSURE PLUS® HN

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

## Patient Conditions:

- For patients with increased calorie and protein needs, such as following surgery or injury.
- For patients with limited volume tolerance and early satiety.

## Features:

- For supplemental or total nutrition
- For oral or tube feeding
- 1.5 CaVmL,
- High nitrogen
- Calorically dense

## Ingredients:

Vanilla: -D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate,

Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

G. ENSURE® POWDER:

Usage: ENSURE POWDER (reconstituted with water) is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- For patients who need a low-residue diet

Features:

- Convenient, easy to mix
- Low in saturated fat
- Contains 9 g of total fat and < 5 mg of cholesterol per serving
- High in vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy

Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

The fat source is corn oil.

Corn oil	100%
----------	------

Carbohydrate:

ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

Vanilla:



Corn Syrup	35%
Maltodextrin	35%
Sucrose	30%

#### H. ENSURE® PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

#### Patient Conditions:

- For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
- For patients with swallowing impairments

#### Features:

- Rich and creamy, good taste
- Good source of essential vitamins and minerals
- Convenient-needs no refrigeration
- Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

#### Ingredients:

Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate,

Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is nonfat milk.

Nonfat milk	100%
-------------	------

Fat:

The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil	100%
--------------------------	------

Carbohydrate:

ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors:

Sucrose	56%
Lactose	27%
Modified food starch	17%

## Chocolate:

Sucrose	58%
Lactose	26%
Modified food starch	16%

## I. ENSURE® WITH FIBER:

Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

## Patient Conditions:

-For patients who can benefit from increased dietary fiber and nutrients

## Features:

- New advanced formula-low in saturated fat, higher in vitamins and minerals
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Good source of fiber
- Excellent source of essential vitamins and minerals
- For low-cholesterol diets
- Lactose- and gluten-free

**Ingredients:**

Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride, Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

**Protein:**

The protein source is a blend of two high-biologic-value proteins-casein and soy.

Sodium and calcium caseinates	80%
Soy protein isolate	20%

**Fat:**

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

High-oleic safflower oil	40%
Canola oil	40%

Corn oil 20%

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of  $\leq 30\%$  of total calories from fat,  $< 10\%$  of the calories from saturated fatty acids, and  $\leq 10\%$  of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Maltodextrin	66%
Sucrose	25%
Oat Fiber	7%
Soy Fiber	2%

Chocolate:

Maltodextrin	55%
Sucrose	36%
Oat Fiber	7%
Soy Fiber	2%

#### Fiber:

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl. oz can. The ratio of insoluble to soluble fiber is 95:5.

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

#### J. Oxepa<sup>TM</sup> Nutritional Product

Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil),  $\gamma$ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

#### Caloric Distribution:

Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs. The distribution of Calories in Oxepa is shown in Table IV.

Table IV. Caloric Distribution of Oxepa

	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)	25	105.5	28.1
Protein (g)	14.8	62.5	16.7

Water (g)	186	785	---
-----------	-----	-----	-----

**Fat:**

-Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).

-The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table V.

-Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table VI.

-Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

Table V. Typical Fatty Acid Profile

	% Total	g/8 fl oz*	g/L*
Fatty Acids			
Caproic (6:0)	0.2	0.04	0.18
Caprylic (8:0)	14.69	3.1	13.07
Capric (10:0)	11.06	2.33	9.87
Palmitic (16:0)	5.59	1.18	4.98
Palmitoleic	1.82	0.38	1.62
Stearic	1.94	0.39	1.64
Oleic	24.44	5.16	21.75
Linoleic	16.28	3.44	14.49
$\alpha$ -Linolenic	3.47	0.73	3.09
$\gamma$ -Linolenic	4.82	1.02	4.29
Eicosapentaenoic	5.11	1.08	4.55
n-3-Docosapent- aenoic	0.55	0.12	0.49
Docosaheptaenoic	2.27	0.48	2.02
Others	7.55	1.52	6.72

Fatty acids equal approximately 95% of total fat.



Table VI. Fat Profile of Oxepa.

% of total calories from fat	55.2
Polyunsaturated fatty acids	31.44 g/L
Monounsaturated fatty acids	25.53 g/L
Saturated fatty acids	32.38 g/L
n-6 to n-3 ratio	1.75:1
Cholesterol	9.49 mg/8 fl oz
	40.1 mg/L

**Carbohydrate:**

-The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).

-The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.

-The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO<sub>2</sub>) production. High CO<sub>2</sub> levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.

-Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The carbohydrate level in Oxepa

is also sufficient to minimize gluconeogenesis, if energy needs are being met.

Protein:

-Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).

-The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.

-Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on CO<sub>2</sub> production, a high protein diet will increase ventilatory drive.

-The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.

- The amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by the National Academy of Sciences.

\* Oxepa is gluten-free.

CLAIMS:

1. An isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence represented by SEQ ID NO:1 (Figure 12).
2. The isolated nucleotide sequence of claim 1 wherein said sequence is represented by SEQ ID NO:1.
3. The isolated nucleotide sequence of claims 1 or 2 wherein said sequence encodes a functionally active desaturase which utilizes a polyunsaturated fatty acid as a substrate.
4. The nucleotide sequence of claim 1 wherein said sequence is derived from a mammal.
5. The nucleotide sequence of claim 4 wherein said Sequence is derived from a human.
6. A purified protein encoded by said nucleotide sequence of claims 1 or 2.
7. A purified polypeptide which desaturates polyunsaturated fatty acids at carbon 5 and has at least about 50% amino acid similarity to the amino acid sequence of said purified protein of claim 6.

8. A method of producing a human  $\Delta 5$ -desaturase comprising the steps of:

- a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 12);
- b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of said human  $\Delta 5$ -desaturase.

9. The method of claim 8 wherein said host cell is a eukaryotic cell or a prokaryotic cell.

10. The method of claim 9 wherein said prokaryotic cell is selected from the group consisting of E. coli, cyanobacteria, and B. subtilis.

11. The method of claim 9 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

12. The method of claim 11 wherein said fungal cell is a yeast cell.

13. The method of claim 12 wherein said yeast cell is selected from the group consisting of Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. and Pichia spp.

14. The method of claim 13 wherein said yeast cell is Saccharomyces cerevisiae.
15. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 12) operably linked to b) a promoter.
16. A host cell comprising said vector of claim 15.
17. The host cell of claim 16, wherein said host cell is a eukaryotic cell or a prokaryotic cell.
18. The host cell of claim 17 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.
19. The host cell of claim 17 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.
20. The host cell of claim 19 wherein said fungal cell is a yeast cell.
21. The host cell of claim 20 wherein said yeast cell is selected from the group consisting of Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. and Pichia spp.

22. The host cell of claim 21 wherein said host cell is Saccharomyces cerevisiae.
23. A plant cell, plant or plant tissue comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell or tissue.
24. The plant cell, plant or plant tissue of claim 23 wherein said polyunsaturated fatty acid is AA or EPA.
25. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 23.
26. A transgenic plant comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.
27. A mammalian cell comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of altered levels of AA or EPA when said cell is grown in a culture media comprising a fatty acid selected from the group consisting of an essential fatty acid, LA and ALA.
28. A transgenic, non-human mammal whose genome comprises a DNA sequence encoding a human  $\Delta 5$ -desaturase operably linked to a promoter.

29. The transgenic, non-human mammal of claim 28, wherein said DNA sequence is represented by SEQ ID NO:1 (Figure 12).

30. A fluid produced by said transgenic, non-human mammal of claim 29 wherein said fluid comprises a detectable level of at least human  $\Delta 5$ -desaturase.

31. A method for producing a polyunsaturated fatty acid comprising the steps of:

- a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 12);
- b) constructing a vector comprising said isolated nucleotide sequence;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of said human  $\Delta 5$ -desaturase enzyme; and
- d) exposing said expressed human  $\Delta 5$ -desaturase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

32. The method according to claim 31, wherein said substrate polyunsaturated fatty acid is DGLA or 20:4n-3 and said product polyunsaturated fatty acid is AA or EPA, respectively.

33. The method according to claim 31 further comprising the step of exposing said product polyunsaturated fatty acid to an elongase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

34. The method according to claim 33 wherein said product polyunsaturated fatty acid is AA or EPA and said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid, respectively.

35. The method of claim 33 further comprising the steps of exposing said another polyunsaturated fatty acid to an additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

36. The method of claim 35 wherein said final polyunsaturated fatty acid is (n-6)-docosapentaenoic acid or docosahexaenoic (DHA) acid.

37. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33, and said final polyunsaturated fatty acid produced according to the method of claim 35.

38. The nutritional composition of claim 37 wherein said product polyunsaturated fatty acid is AA or EPA.

39. The nutritional composition of claim 37 wherein said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid.



40. The nutritional composition of claim 37 wherein said final polyunsaturated fatty acid is DHA.

41. The nutritional composition of claim 37 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

42. The nutritional composition of claim 41 wherein said nutritional composition is administered to a human or an animal.

43. The nutritional composition of claim 42 wherein said nutritional composition is administered enterally or parenterally.

44. The nutritional composition of claim 41 wherein said nutritional composition further comprises at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and protein hydrolysates.

45. The nutritional composition of claim 44 wherein said nutritional composition further comprises at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

46. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33, and said final polyunsaturated fatty acid produced according to the method of claim 35 and 2) a pharmaceutically acceptable carrier.

47. The pharmaceutical composition of claim 46 wherein said pharmaceutical composition is administered to a human or an animal.

48. The pharmaceutical composition of claim 46 wherein said pharmaceutical composition further comprises an element selected from the group consisting of a vitamin, a mineral, a carbohydrate, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

49. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33 and said final polyunsaturated fatty acid produced according to the method of claim 35.

50. The animal feed of claim 49 wherein said

product polyunsaturated fatty acid is AA or EPA.

51. The animal feed of claim 49 wherein said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid.

52. The animal feed of claim 49 wherein said final polyunsaturated fatty acid is (n-6)-docosapentaenoic acid or DHA.

53. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33 and said final polyunsaturated fatty acid produced according to the method of claim 35.

54. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 37 in an amount sufficient to effect said treatment.

55. The method of claim 11 wherein said insect cell is Spodoptera frugiperda.

56. The method of claim 19 wherein said insect cell is Spodoptera frugiperda.

1/40

Sections of the Desaturases	Clone ID from		Keyword
	Incyte	Lifeseq Database	
151-300 delta 5	3808675	fatty acid desaturase	
301-446 delta 5	354535	delta 6	
151-300 delta 6	3448789	delta 6	
151-300 delta 6	1362863	delta 6	
151-300 delta 6	2394760	delta 6	
301-457 delta 6	3350263	delta 6	

Figure 1

2/40

Edited Contig 2692004

GCACGCCGACCGGCGGGAGATCCTGGCAAGATATCCAGAGATAAAGTCCTTGATGAACCTGATCCCAATTTGATATGGATTATATTA  
TCA'TGGT'TCTCACCCAGTTGGTGCA'TTTACATAGTAAAGACTTGGACTGGAANTGGTCATATTTGGGGCCCTATGCGTTTGGCAGTTGC  
ATTAAACCACTCAATGACTCTGGCTATTCATGAGATTGCCCAATGGTCCCTTTGGCAACTGCAAGCAATGTGGAATCGCTGGTTTGGAAAT  
GTTTGC'TAATCTTCCTATTGGGATTCCATATTCAATTTCC'TTAAGAGGTATCACATGGATCATCATCGGTACCTTGGAGCTGATGGCGTCG  
ATGTAGATA'TTCC'TACCGATT'TTGAGGGCTGGTCTTCTGTACCGCTTTCAGAAAGTTTATATGGGTATTCTTCAGCCTCTCTTTTATGCC  
TTTCGACCTCTGTTTCATCAACCCCAACCAATTACGTATCTGGAAGTTATCAATACCGTGGCACAGSTCAC'TTTTGACATTTTAAATTTATTA  
CTTT'TGGGAATTAATCCCTTAGTCTACATGTTGGCAGCATCTTACTTGGCCT'GGGT'TTGACCCCAATTTCTGGACATTTTATAGCTGAGC  
ATTACNTGTTCTTAAAGGGTCATGAACCTTACTCATATTATGGGCTCTGAATTTACTTACCTTCAATGTGGGTATCATATGAACATCAT  
GAT'TCCCCAACATTCCTGGAAAAAGTCTTCCACTGGTGAGGAAATAGCAGCTGAATACTATGACAACTCCCTCACTACAATTCCTGGAT  
AAAAGTACTGTATGATTTTGTGATGGATGATACAAATAGTCCCTACTCAAGAATGAAGAGGCCAACAAAGGAGAGATGGTGTGGAGTAAA  
TAT'CA'TTAGTGCCRAAGGATTC'TTCCAAACTTTAGATGATAAAATGGAATTTTTCATTATTAACCTTGAGACCAAGTGTGCTCAGAA  
GCTCCCTGGCACAA'TTTCAGAGTAAGAGCTCGGTGATACCAAGAAGTGAATCTGGCTTTTAAACAGTCAGCCTGACTCTGTACTGCTCAGT  
TTCAC'TCACAGGAACTTGTGACTTGTGTATTATCGTCA'TTGAGGATGTTTTCAC'CATGCTGTCA'TTTAT'AAAGCA'ATCA'TTT'AAAAAGC  
TTC'AAAAAGCTATTTCCGCCAGG

Figure 2

3/40

Edited Contig 2153526

TTACCTTCTAGTCCGCTTCTTCCTCACTTATGTGCCACTATTGGGGCTGAAAGCTTCCTGGGCCCTTTCCTTCATAGTCAGGTTCCTGGANA  
GCAACCTGGTTTGTGTGGTGACACAGATGAACCATATTTCCCATGCACATTGATCATGACCGGAACATGGACTGGGTTCACACCCAGCTCCAG  
GCCACATGCAATGTCCACRAGTCTGCCCTTCAATGACTGGTTCACTGGACACCTCAACTTCCAGATTGAGCAGCATCTTTTCCCAACGATGCC  
TTCCAGACAAATTACCCACAAGTGGCTCCCTGGTGGCAGTCCCTTTGTGTGCCAAGCATGGCATAGAGTACCAGTCCAAAGCCCCCTGCTGTCAAGCCT  
TCGCCGACATCCACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCCATATCTTCAACCAATAACAACAGCCACCCCTGCCCACTCTGG  
AAGAAGAGGAGGAAGACTCTGGAGCCAAAGGCAGAGGGGAGCTTGAGGGACAATGCCACTATAGTTTAACTACAGAGGGGTGGGTTCGGG  
GACATAAAGCCTCTGACTCAAACTCCTCCCTTTTATCTTCTAGCCACAGTTCTAAGACCCAAAGTGGGGGTGGACACAGAAAGTCCCTATAGA  
GGGAAGGAGCT

Figure 3

4/40

## Edited Contig 3506132

GTCTTTTACTTTGGCAATGGCTGGATTCCCTACCCCTCATCAGGCCTTTGTCCCTTGCTACCTCTCAGGCCCAAGCTGGATGGCTGCAACATGA  
TTATGGCCACCTGTCTGTCTACAGAAAACCCCAAGTGGAAACCACTTGTCACAAATTCTGTCATTGGCCACTTAAAGGGTGCCTCTGCCAACT  
GGTGGAAATCATCGCCACTTCCAGCACCCACGCCCAAGCCTAACATCTTCCACAAGGATCCCGATGTGAACATGCTGCACCGTTTGTCTTGGGC  
GAATGGCAGCCCATCGAGTACGGCAAGA

Figure 4

5/40

Edited Contig 3854933

CAGGACCTACCCCGCGCTACTTCACCTGGGAGGAGGTGGCCACGGCTCAGGGTCCGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTA  
CAACATCAGCGAGTTACCCGCGGCTCCCGGGTCAATCAGCCACTACGCCGGCAGGATGCCACGGATGCCCTTTGTGGCCT  
TCCACATCAACAAGGGCCTTGTAAGAATATGAACCTCTCTGATTTGGAGAACTGTCTCCAGAGCAGCCAGCTTTGAGCCCAACCAAG  
AATAAGAGCTGACAGATGAGTTCGGGGAGCTGCGGGCCACAGTGGAGCGGATGGGCTCATGAAGGCCAACCATGTCTTCTTCTGCTGTA  
CCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCCCTTGGGCTTTGGGACGTCTTTTGGCCCTTCCCTCTGTGGGTGC  
TGCTCAGTGCAGTTCAGGCCCAGGCTGGCTGGCTGCAGCATGACTTTGGGCACTGTCTGCTTCAGCACCTCAAGTGAACCATCTGCTA  
CATCATTTTGTGATTGGCCACCTGAAGGGGCCCCCGCCAGTTGGTGGAAACACATGCACCTCCAGCACCATGCCAAGCCCACTGCTTCCG  
CAAGACCCAGACATCAACATGCATCCCTTCTTTGCTTGGGGAAGATCCTCTCTGTGGAGCTTGGGAACAGAAAAAATATATGC  
CGTACAAACCAACAGCACARATACCTTCTTAATTGGGCCCCCAGCCCTTGTGCTCTCTACTTCCAGTGGTATATTTCTATTGTGTTATC  
CAGCGAAAGAGTGGGTGGACTTGGCCTGGATCAGCAACAGGAATACGATGAAGCCGGCTTCCATTGTCCACCGCAANTGCTTCTTAA

Figure 5





7/40

Contig 2535

GTCTTTTACTTTGGCAATGGCTGGATTCTACCCCTCATCAGGGCCCTTGTCTTCTGCTACCTCTCAGGCCCAAGCTGGATGGC  
TGCATCATGATATGGCCACCTGTCTGTCTACAGAAACCCAAAGTGGAAACCCCTTGTCCACAATTCGTTCATTGGCCACT  
TAAAGGCTGCTCTGCCAACTGGTGGATCACTGCTTCCAGCACACGGCAAGCCCTAACATCTCCACAAGGATCC  
GATGTAACTATGCTGACCTGTGTTGTTCTGGGCAATGGCAGCCATCGAGTACGGCAAGAAAGAAATACCTGCCC  
CTACATTCACCCAGCAGAAATACCTTCTTCTGATTTGGCCGCTGCTCATCCCATGTATTCAGTACCAATCATCATG  
ACCATGATCGTCCATAAGAACTGGGTGGACCTGGCCCTGGCTGCTCATCCCATGTATTCAGTACCAATCATCATG  
TCTACGGCATCCCTGGGAGCCCTCCCTTCTCACTTCACTTCACTTCACTTCACTTCACTTCACTTCACTTCACTTCACTT  
ATGATTCACATCGTCTATGGAGATTGACCAAGAGGCTTACCTGACTGGTTCAGTACGCACTTCACTTCACTTCACTTCACTT  
GGAGCAGTCTCTTCAACGACTGGTTCAGTGGAGCCCTTAACTTCACTTCACTTCACTTCACTTCACTTCACTTCACTT  
GACAACTTACACAAGATCGCCCGCTGGTGAAGTCTCTATGTGCCAAGCATGGCATTTGAATACCAAGGAGAAAGCCCTTAC  
TGAGGGCCCTGCTGGACATCATCAGGTCCCTGAAGAAGTCTGGGAAGCTGTGGTGGATGGCCAGAGGAATGATGGGCTTTGTCTGAGGG  
CACAGCCCCCGGACACCGTGGGAAGGGTGCAGGTGGGTGATGGCCAGAGGAATGATGGGCTTTGTCTGAGGG  
CTGTCTCGAGAGGCTGGTATGCACTGTCTACGAGACCCCTATGGGAACCTTCCCTCCCTCAGCCCTCAGCCATCAGCCATGCCCAGTGGCTC  
ATCTCTCCCTATAGCACCTTGGCTCAGGAGAGGTGGCCACCGGGGGTGGCTGTCTGCTTACCTTACCTTACCTTACCTTACCTT  
CTAGCTCCCTTCTTCCAGGAGCAGAGAGGTGGCCACCGGGGGTGGCTGTCTGCTTACCTTACCTTACCTTACCTTACCTT  
GAGAGGAGAC'CAAGGGTCTATGGGTCTGGGCTGTGAGTCTTCCCTTTCAGCTGGTTCATAGGCTGGGAGGCTTACCTTACCTTACCTT  
TCTTACAGTGTCTTGGGTTTCATAGGGGAGGCTTCTATAGAGAGGCTTCTTGTATACAAAGCTGGGTCTTCCCTTCCCTTACCTTCTC  
CTTACAGGCTGCTTGGTCCACCTTTCATAGAGAGGCTTCTTGTATACAAAGCTGGGTCTTCCCTTCCCTTACCTTCTC  
TAACTACCCGAGGCTCTCTTAAAGTGTCCAGGCTTCTTGTATACAAAGCTGGGTCTTCCCTTCCCTTACCTTCTC  
GATCTCTCCACCCCATCACTAGAGTGTCTTGTATACAAAGCTGGGTCTTCCCTTCCCTTACCTTCTC  
TGACCTTGGGACCAAGGGGAGTCCCTGCTCTTGTATACAAAGCTGGGTCTTCCCTTCCCTTACCTTCTC  
TGGCTTGGAGGCTCAGCCCCACCTCCAGCTTCTTCTCAGGCTGCTTCCCTTCCCTTCCCTTACCTTCTC  
CTAAAGGCTCTGTATACAGTGGGAGTGGCAGGCTTCCCTTCCCTTCCCTTACCTTCTC

Figure 7



9/40

## FastA Match of Ma29 and contig 253538a

SCORES Initl: 117 Initn: 225 Opt: 256  
 Smith-Waterman score: 408; 27.0% identity in 441 aa overlap

```

Ma29.pep      10      20      30      40      50
MGTDQGKT---FTWEELAAHNTKDDLLLAIRGRVYDVKFLSRHPGGVDTL LLGAGRDVT
253538a      10      20      30      40      50
QGPTPRYFTWDEVAQRS GCCEERWLVIDRKVYNISEFTRRHPGGS RVISHYAGQDAT

Ma29.pep      60      70      80      90     100     110
PVFEMYHAF-GAADAIMKKYVVGTLVSNELPIFPEPTVFHKTIKTRVEGYFTDRNIDPKN
253538a      60      70      80      90     100     110
DPFVAFHINKGLVKKYMNSLLIGEL-SPEQPSF-EPTKNKELTDEFREL RATVERMGLMK

Ma29.pep     120     130     140     150     160     170
RPEIWGRYALIFGSLIASYYAQLFVPFVVERTWLQVVF-AIIMGFACAQVGLNPLHDASH
253538a     120     130     140     150     160     170
ANHVF--FLLYLLHILLLDGAAWLTLWVFGTSFLPFLLCVLLSAVQAQAGWLQ-HDYGYH

Ma29.pep     180     190     200     210     220
FSVTHNPTVWKILGATHDF----FNGASYLVWMYQHMLGHHPTYNIAGADPDVSTSE---
253538a     180     190     200     210     220
LSVYRKPK-WNHL--VHKFVIGHLKGASANWNNHRH-FQHHAKPNIFHKDPDVNMLHVFV

Ma29.pep     230     240     250     260     270     280
----PDVRRIKPNQKWF-VNHNQHMFV--PFLYGLLAFKVRIQDINILYFVKTNDAIRV
253538a     230     240     250     260     270     280
LGEWQPIEYCKKLLYLPYNHQUEYFFLIGPPLLIPMYFOYQI----IMTIVHKMWDLDL

Ma29.pep     290     300     310     320     330     340
NPISTWHTVMFWGGKAFFVWRLIVPLQYLPGLKVL LFTVADMVSSYWLALTFQANHVV
253538a     290     300     310     320     330     340
----AWAVSYYI---RFFITY---IPF-YGILG-ALLFLNFIRFLESHWFWVTQMNHIV

Ma29.pep     350     360     370     380     390
EEVQWPLPDENGIIQKDWAAQVETT----QDYAHDShLWTSITGSLNYQAVHHLFPNVS
253538a     350     360     370     380     390
MEI-----DQEAY--RDWFSSQLTATCNVEQSFFND---WFS--GHLNFQIEHHLFPTMP

Ma29.pep     400     410     420     430     440
QHHPDILAIKNTCSEYKVPYLVKDTFNQAFASHLEHLRVLGLRPKEEX
253538a     400     410     420     430     440
RHNLHKIAPLVKSLCAKHGIEYQEKPLLRALLDIIRSLKKSGLWLDAYLHKX
380      390      400      410      420      430

```

Figure 9

10/40

## FastA Match of Ma524 and contig 253538a

SCORES Initl: 231 Initn: 499 Opt: 401

Smith-Waterman score: 620; 27.3% identity in 455 aa overlap

```

      10      20      30      40      50      59
Ma524.pep  MAAAPSVRTFTRAEVLNAEALNEGKKDAEAPFLMIIDNKVYDVREFVPDHPGGSVILTH-
      | : | | | | | | | | | | | | | | | | | | | | | | | | | | | |
253538a    QGPTPRYFTWDEV-----AQRSGCEERWLVIDRKVYNISEFTRRHPPGGSRVISHY
      10      20      30      40      50

      60      70      80      90      100     110
Ma524.pep  VGKDGTDVFDTFHPEAAW--ETLANFYVGDIIDE---SDRDIKNDDFAAEVRKLRTLFQSL
      | : | : | | | | | | | | | | | | | | | | | | | | | | | | | |
253538a    AGQDATDPFVAFHINKGLVKYMNSSLIGELSPEQPSFEPTKNKELTDEFRELRTATVERM
      60      70      80      90      100     110

      120     130     140     150     160     170
Ma524.pep  GYYDSSKAYYAFKVSEFNLCTWGLSTVIVAKWGQTSTLANVLSAALLGLFWQCGWLAHDF
      | : : : : : : : : : : : : : : : : : : : : : : | | | | | |
253538a    GLMKANHVFLLYLLHILLDGAAWLTLWVFG-TSFLPFLLCVLLSAVQAQAGWLQHDY
      120     130     140     150     160

      180     190     200     210     220     230
Ma524.pep  LHHQVFQDRFWGDLFGAFLGGVCQGFSSSWWKDKHNTTHAAPNVHGEDPDIDTHPLLTWS
      | : : : : : : : | : | : | : | : | : | : | : | : | : | : |
253538a    GHLSVYRKPKWNHLVHKFVIGHLKGASANWNNHRHFQHHAKPNIFHKDPDVN---ML---
      170     180     190     200     210     220

      240     250     260     270     280     290
Ma524.pep  EHLEMFSDVPDEELTRMWSRFMVLNQTFWYFPILS---FARLSWCLQSILFVLPNGQAH
      | : : : : : : : | : : : : | : : : : | : : : : | : : : : |
253538a    -HVF-VLGEWQPIEYGKKKLKYLPNHQHEYFFLIGPPLLIPMYFQYQIIMTMI----VH
      230     240     250     260     270

      300     310     320     330     340     349
Ma524.pep  KPSGARVPISLVEQLSLAMHWTWYLAITMFLFIK--DPVNMLVYFLVSQAVCGNLLAIVFS
      | : : : : : : : | : : : : | : : : : | : : : : | : : : : |
253538a    K-----NWVDLAWAVSYIRFFITYIPFYGILGALLFLNFIREFLESHWFVWVWVQ
      280     290     300     310     320

      350     360     370     380     390     400     409
Ma524.pep  LNHNGTPVISKEEAVDMDFFTKQIITGRDVHPGLFANWFTGGLNYQIEHHLFSPMPRHNF
      | : | : : : | | : : : : : : : : : : | : | : | : | : | : | :
253538a    MNHITMEI--DQEAAYR-DWFSQLTATCNVEQSFNDWFSCHLNFQIEHHLFPTMPRHNL
      330     340     350     360     370     380

      410     420     430     440     450
Ma524.pep  SKIQFAVETLCKKCNVRYHTTGMIETAEVFSRLNEVSKAASKMGKAQX
      | | | | : : | | : : : : : : : : : : | : : : |
253538a    HKIAPLVKSLCAKHGIEYQEKPLLRLALDITRSLKSGKLWLDAYLHKX
      390     400     410     420     430

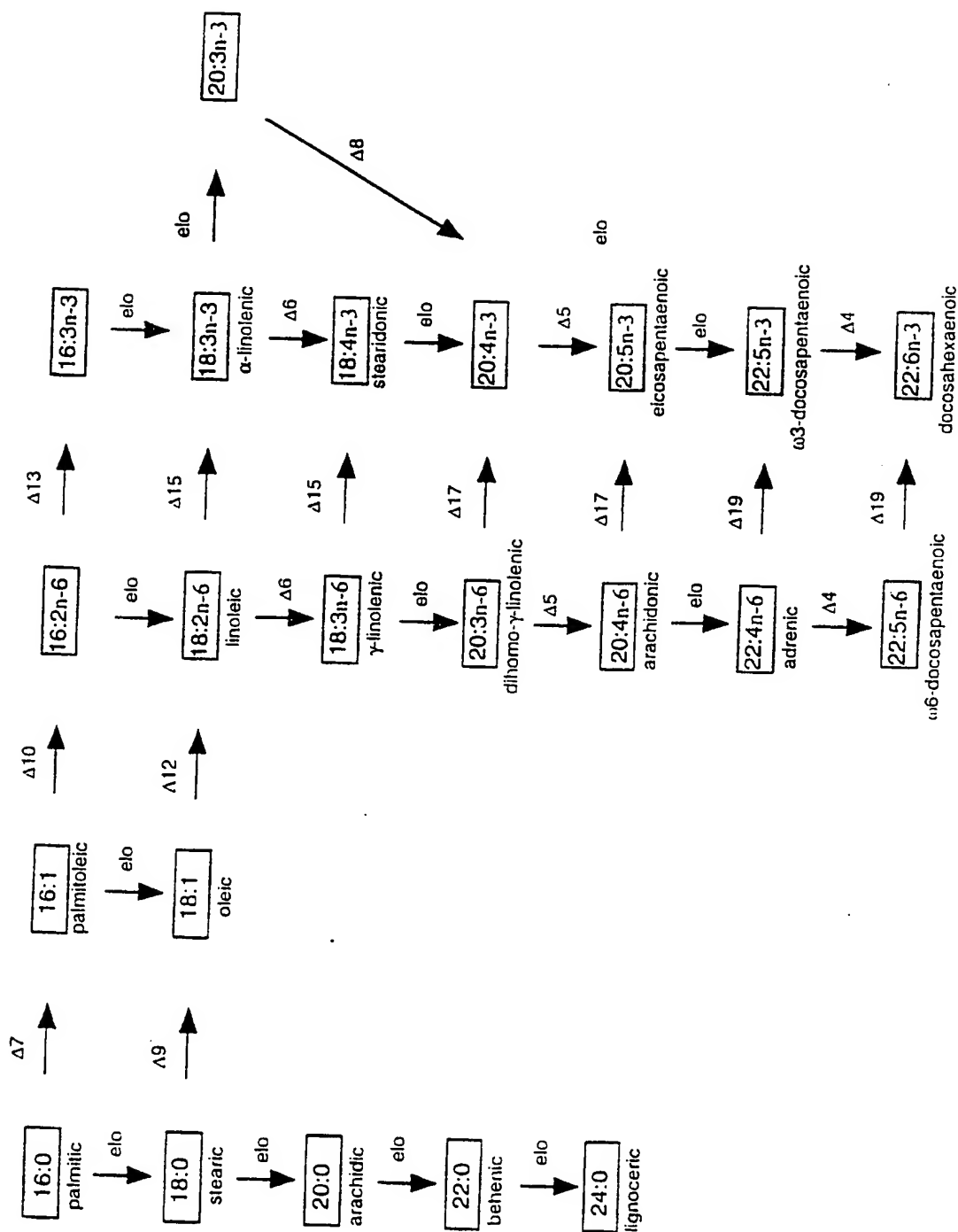
```

Figure 10

11/40

Figure 11

## Fatty Acid Biosynthesis Pathways



12/40

Human  $\Delta 5$ -desaturase

ATGGCCCCGACCCGGTGGCCGCCGAGACCGCGGCTCAGGGACCTACCCCGCGCTACTTCACCTGG  
GACGAGGTGGCCCAGCGCTCAGGGTGCAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAAC  
ATCAGCGAGTTCACCCGCCGCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGAT  
GCCACGGATCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAACTCTCTC  
CTGATTGGAGAACTGTCTCCAGAGCAGCCCAGCTTTGAGCCCAACAAGAATAAAGAGCTGACAGAT  
GAGTTCGGGAGCTGCGGGCCACAGTGGAGCGGATGGGGCTCATGAAGGCCAACCATGTCTTCTTC  
CTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGACGCTGGCTCACCTTTGGGTCCTTGGG  
ACGTCCTTTTGGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCAGTTCAGGCCCAGGCTGGCTGG  
CTGCAGCATGACTTTGGGCACCTGTGCGTCTTCAGCACCTCAAAGTGAACCATCTGCTACATCAT  
TTTGTGATTGGCCACCTGAAGGGGGCCCCGCCAGTTGGTGAACACATGCACTTCCAGCACCAT  
GCCAAGCCCAACTGCTTCCGCAAAGACCCAGACATCAACATGCATCCCTTCTTCTTGGCCTTGGGG  
AAGATCCTCTCTGTGGAGCTTGGGAAACAGAAGAAAAAATATATGCCGTACAACCACCAGCACAAA  
TACTTCTTCTTAATTGGGCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTTCTATTTT  
GTTATCCAGCGAAAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCCTC  
ACTTATGTGCCACTATTGGGGCTGAAAGCCTTCCTGGGCCTTTTCTTCATAGTCAGGTTCCTGGAA  
AGCAACTGGTTTGTGTGGGTGACACAGATGAACCATATTCCCATGCACATTGATCATGACCGGAAC  
ATGGACTGGGTTTCCACCCAGCTCCTGGCCACATGCAATGTCCACAAGTCTGCCTTCAATGACTGG  
TTCAGTGGACACCTCAACTTCCAGATTGAGCACCATCTTTTCCACGATGCCTCGACACAATTAC  
CACAAAGTGGCTCCCTGGTGCAGTCCTTGTGTGCCAAGCGTGGCATAGAGTACCAGTCCAAGCCC  
CTGCTGTCAGCCTTCGCCGACATCATCCACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCC  
TATCTTCACCAATAA

Figure 12

13/40

Human  $\Delta 5$ -desaturase

1 MAPDFVAAET AAQGPTPRYF TWDEVAQRSG CEERWLVIDR KVYNISEFTR  
51 RHPGGSRVIS HYAGQDATDP FVAFHINKGL VKKYMNSLLI GELSPEQPSF  
101 EPTKNKELTD EFRELRAIVE RMGLMKANHV FFLLYLLHIL LLDGAAWLTL  
151 WVFGTSFLPF LLCVLLSAV QAQAGWLQHD FGHLVSFSTS KWNHLLHHFV  
201 IGHILKGAPAS WNNMHFQHH AKPNCFRKDP DINMHPFFFA LGKILSVELG  
251 KQKKKYPYN HQHKYFFLIG PPALLPLYFQ WYIFYFVIQR KKWVDLAWMI  
301 TFYVRFFLTY VPLLGLKAFL GLFFIVRFLE SNWFVWVTQM NHIPMHIDHD  
351 RNMDWVSTQL LATCNVHSA FNDWFSGLN FQIEHHLFPT MPRHNYHKVA  
401 PLVQSLCAKR GIEYQSKPLL SAFADIHSL KESGQLWLDA YLHQ\*

Figure 13



14/40

Contig[0023]  
Sequencher™ "pRAE-7 sequence"

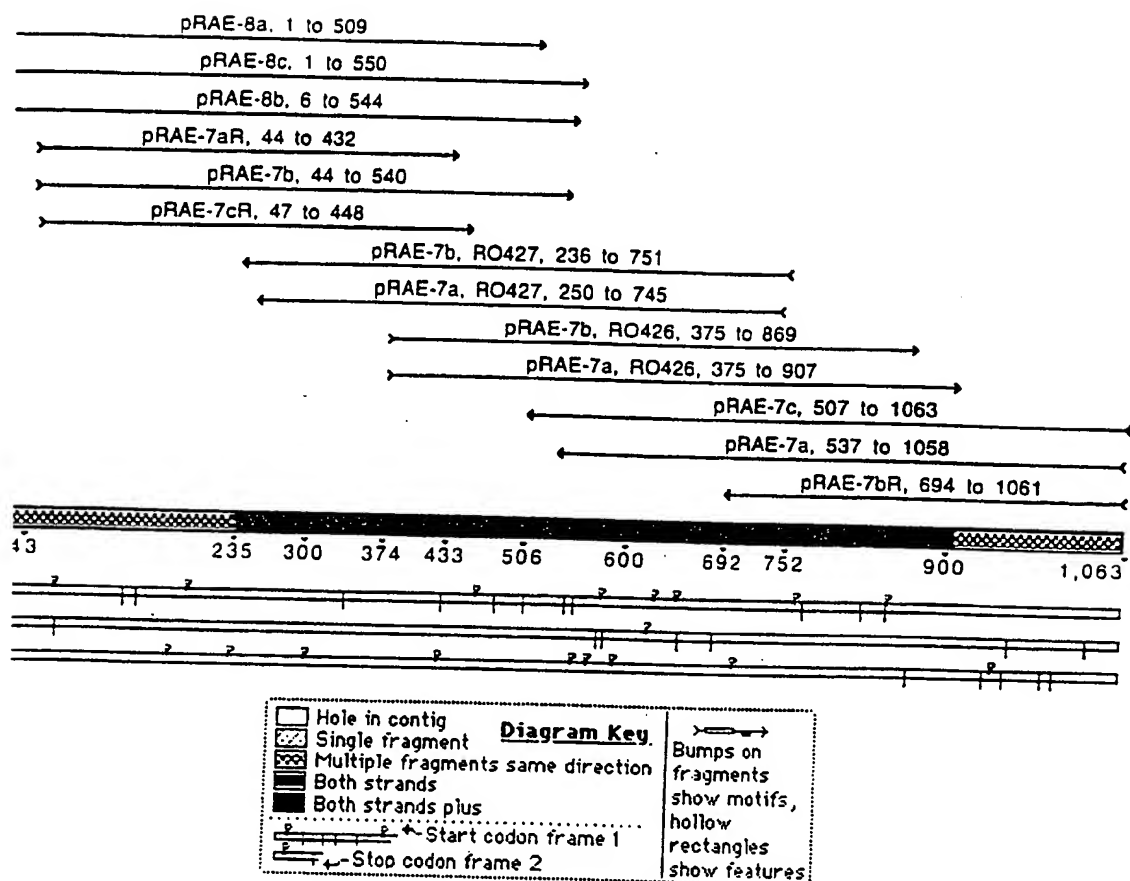


Figure 14

15/40

**pRAE-7 Complete Sequence**

CTC CTG GAG CCC GTC AGT ATC GGC GGA ATT CCG GCA GTT CAG GCC CAG  
Leu Leu Glu Pro Val Ser Ile Gly Gly Ile Pro Ala Val Gln Ala Gln>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

50                  60                  70                  80                  90  
GCT GGC TGG CTG CAG CAT GAC TTT GGG CAC CTG TCG GTC TTC AGC ACC  
Ala Gly Trp Leu Gln His Asp Phe Gly His Leu Ser Val Phe Ser Thr>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

100                110                120                130                140  
TCA AAG TGG AAC CAT CTG CTA CAT CAT TTT GTG ATT GGC CAC CTG AAG  
Ser Lys Trp Asn His Leu Leu His Phe Val Ile Gly His Leu Lys>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

150                160                170                180                190  
GGG GCC CCC GCC AGT TGG TGG AAC CAC ATG CAC TTC CAG CAC CAT GCC  
Gly Ala Pro Ala Ser Trp Trp Asn His Met His Phe Gln His His Ala>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

200                210                220                230                240  
AAG CCC AAC TGC TTC CGC AAA GAC CCA GAC ATC AAC ATG CAT CCC TTC  
Lys Pro Asn Cys Phe Arg Lys Asp Pro Asp Ile Asn Met His Pro Phe>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

250                260                270                280  
TTC TTT GGT TTG GGG AAG ATC CTC TCT GTG GAG CTT GGG AAA CAG AAG  
Phe Phe Ala Leu Gly Lys Ile Leu Ser Val Glu Leu Gly Lys Gln Lys>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

290                300                310                320                330  
AAA AAA TAT ATG CCG TAC AAC CAC CAG CAC AAA TAC TTC TTC CTA ATT  
Lys Lys Tyr Met Pro Tyr Asn His Gln His Lys Tyr Phe Phe Leu Ile>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

340                350                360                370                380  
GGG CCC CCA GGC TTG CTG CCT CTC TAC TTC CAG TGG TAT ATT TTC TAT  
Gly Pro Pro Ala Leu Leu Pro Leu Tyr Phe Gln Trp Tyr Ile Phe Tyr>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

390                400                410                420                430  
TTT GTT ATC CAG CGA AAG AAG TGG GTG GAC TTG GCC TGG ATG ATT ACC  
Phe Val Ile Gln Arg Lys Lys Trp Val Asp Leu Ala Trp Met Ile Thr>  
\_ \_ \_ \_ \_ TRANSLATION OF PRAE-7 MV [ A ] \_ \_ \_ \_ \_>

Figure 15a

16/40

```

      440      450      460      470      480
      *      *      *      *      *
      TTC TAC GTC GGC TTC CTC ACT TAC GTG CCA CTA TTG GGG CTG AAA
      Phe Tyr Val Arg Phe Phe Leu Thr Tyr Val Pro Leu Leu Gly Leu Lys>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

      490      500      510      520
      *      *      *      *
      GCC TTC CTG GGC CTT TTC TTC ATA GTC AGG TTC CTG GAA AGC AAC TGG
      Ala Phe Leu Gly Leu Phe Phe Ile Val Arg Phe Leu Glu Ser Asn Trp>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

      530      540      550      560      570
      *      *      *      *      *
      TTT GTG TGG GTG ACA CAG ATG AAC CAT ATT CCC ATG CAC ATT GAT CAT
      Phe Val Trp Val Thr Gln Met Asn His Ile Pro Met His Ile Asp His>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

      580      590      600      610      620
      *      *      *      *      *
      GAC CGG AAC ATG GAC TGG GTT TCC ACC CAG CTC CAG GCC ACA TGC AAT
      Asp Arg Asn Met Asp Trp Val Ser Thr Gln Leu Gln Ala Thr Cys Asn>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

      630      640      650      660      670
      *      *      *      *      *
      GTC CAC AAG TCT GCC TTC AAT GAC TGG TTC AGT GGA CAC CTC AAC TTC
      Val His Lys Ser Ala Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

      680      690      700      710      720
      *      *      *      *      *
      CAG ATT GAG CAC CAT TTT TTT CCC ACG ATG CCT CGA CAC AAT TAC CAC
      Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Tyr His>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

      730      740      750      760
      *      *      *      *
      AAA GTG GCT CCC CTG CTG CAG TCC TTG TGT GCC AAG CAT GGC ATA GAG
      Lys Val Ala Pro Leu Val Gln Ser Leu Cys Ala Lys His Gly Ile Glu>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

      770      780      790      800      810
      *      *      *      *      *
      TAC CAG TCC AAG CCC CTG CTG TCA GCC TTC GCC GAC ATC ATC CAC TCA
      Tyr Gln Ser Lys Pro Leu Leu Ser Ala Phe Ala Asp Ile Ile His Ser>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

      820      830      840      850      860
      *      *      *      *      *
      CTA AAG GAG TCA GGG CAG CTC TGG CTA GAT GCC TAT CTT CAC CAA TAA
      Leu Lys Glu Ser Gly Gln Leu Trp Leu Asp Ala Tyr Leu His Gln ***>
      _a_a_a_a_a_TRANSLATION OF PRAE-7 MV (A) _a_a_a_a_a_>

```

Figure 15b

17/40

**FastA Match of the Gene in pRAE-7 and the *M. alpina*  
 $\Delta 5$ -desaturase (Ma29) Translated Sequences**

SCORES      Init1:    62    Initn:    105    Opt:    245  
 Smith-Waterman score: 271;    28.4% identity in 292 aa overlap

```

pRAE-7.pep          10      20      30      40
                    LLEFVSIGGIPAVQAQAGWLQ-HDFGHLSV-FSTSKWNHL--LH
Ma29.pep            ASYYAQLFVPFVVERTWLQVVFALIMGFACAQVGLNPLHDASHFSVTHNPTVWKILGATH
                    140      150      160      170      180      190

pRAE-7.pep          50      60      70      80      90
                    HFVIGHLKGAFASWWNHMH-FQHHAKPNCFRKDPDINM-HPFFFFALGKILSVELGKQKKK
Ma29.pep            DF-----FNGASYLVWMYQHMLGHHPTNIAGADPDVSTSEP-----DVRRIKPNQK
                    200      210      220      230

pRAE-7.pep          100     110     120     130     140     149
                    YMPYNH--QHKYF-FLIGPPALLPLYFQWYIFYFVIQ----RKKWVDLAWMITFY--VRF
Ma29.pep            WF-VNHINQHMFPFLYGLLAFKVRIQDINILYFVKINDAIRVNPISWTWHTVMFWGGKAF
                    240     250     260     270     280     290

pRAE-7.pep          150     160     170     180     190
                    FLTY---VPL--LGLKAFLGLFFIVRFLESNWFVWVTQMNHIPMHID---HDRN---MD
Ma29.pep            FWYRLIVPLQYLPLGKVLLLFTVADMVSSYWLALTFQANHVVVEVQWPLPDENGIIQKD
                    300     310     320     330     340     350

pRAE-7.pep          200     210     220     230     240     250
                    WVSTQLQATCN-VHKSAFNDWFSGHILNFQIEHHLFFTPMRHNYHGWAPLVQSLCAKHGIE
Ma29.pep            WAAMQVETTQDYAHDShLWTSITGSLNYQAVHHLFPNVSQHHPDILAIKNTCSEYKVP
                    360     370     380     390     400     410

pRAE-7.pep          260     270     280
                    YQSKPLL-SAFADIIHSLKESGQLWLDAYLHQX
Ma29.pep            YLVKDTFWQAFASHLEHLRVLGLRPKEEX
                    420     430     440

```

Figure 16

18/40

**FastA Match of the Gene in pRAE-7 and *M. alpina*  
 $\Delta$ 6-desaturase (Ma524) Translated Sequences**

SCORES      Init1: 278 Initn: 483 Opt: 301  
 Smith-Waterman score: 498;      31.9% identity in 285 aa overlap

pRAE-7.pep		10	20	30	40		
		LLEPVSIGGIPAVQAQAGWLQHDGHLVSTSKWNHLLHHFVIG					
Ma524.pep	GLSTVIVAKWGQTSTLANVLSAALLGLFWQCGWLAHDFLHHQVFQDRFWGDLFGAFLGG						
	140      150      160      170      180      190						
pRAE-7.pep		50	60	70	80	90	
		HLKGAPASWWNHMFQHHAKPNCFRKDPDINMHPFF---FALGKILSV---ELGKQKKK					
Ma524.pep	VCQGFSSSWWKDKHINTHHAAPNVHGEDPDIDTHPLLTWSEHALEMFSQVDPDEELTRMWSR						
	200      210      220      230      240      250						
pRAE-7.pep		100	110	120	130	140	
		YMPYNHQHKYFFLIGPPALLPLYFQWYIFYFV-----IQRKKWVDLAWMITF					
Ma524.pep	FMVLN-QTWFFYPILSFARLSWCLOSILFVLPNGQAHKPSGARVPISLVEQLSLAMHWITW						
	260      270      280      290      300      310						
pRAE-7.pep		150	160	170	180	190	200
		YVRFFLTIV--PLLGLKAFLGLFFIVRFLESNWFVWVTQMNHHPMI---DHDRNMDWVS					
Ma524.pep	YLATMFLFIKDPV---NMLVYFLVSQAVCGNLLAIVFSLNHNMGMPVISKEEAVDMDFFT						
	320      330      340      350      360      370						
pRAE-7.pep		210	220	230	240	250	260
		TQLQATCNVHKSAFNDWFSGHLNFQIEHHLFFTPPFHNYHK/APLVQSLCAKHGIEYQSK					
Ma524.pep	KQIITGRDVHFGFLFANWFTGGLNFIQIEHHLFFSMPFHNFYSKIQPAVETLCKKYNVRYHTT						
	380      390      400      410      420      430						
pRAE-7.pep		270	280				
		PLLSAFADIHSLKESGQLWLDAYLHQX					
Ma524.pep	GMIEGTAEVFSRLNEVSKAASKMGAQX						
	440      450						

Figure 17

19/40

### FastA Match of the Gene in pRAE-7 and contig 2535

SCORES Initl: 1028 Initn: 1424 Opt: 1430  
Smith-Waterman score: 1430; 71.0% identity in 276 aa overlap

```
pRAE-7.pep      10          20          30          40          50
                  LLEFVSIGGIPAVQAQAGWLQHDFGHLSVVFSTSKWNHLLHHFVIGHLKGAPA
                |||||:|||:|||:|||:|||:|||:|||:|||:|||:|||:|||
2535            VFYFGNGWIPTLTAFVLATSQAQAGWLQHDYGHLSVVRKPKWNHLVHKFVIGHLKGASA
                10          20          30          40          50          60

pRAE-7.pep      60          70          80          90          100         110
                  SWNNHMHFOHHAKEPNCFRKDPPDINM--HPFFALGKILSVELGKQKKKPYNHNHQHYFFL
                |||||:|||:|||:|||:|||:|||:|||:|||:|||:|||:|||
2535            NWWNHRHFQHHAKENIFHKOPDVNMLH--VFVLGEWQPIEGYKKKLKYLPNHNQHEHYFFL
                70          80          90          100         110

pRAE-7.pep      120         130         140         150         160         170
                  IGPPALLPLYFQWYIFYFYVIQRKKWVDLAWMITFYVRFFLTIVPLLG-LKAFLGLFFIVR
                |||||:|||:|||:|||:|||:|||:|||:|||:|||:|||:|||
2535            IGPPLLIPMYFOYQIIMTMIVHKNWVDLAWAVSYIRFFITYIPFYGILGALLFLNFIR
               120        130        140        150        160        170

pRAE-7.pep      180         190         200         210         220         230
                  FLESNWFWVWTQMNHIPMHIDHDRNDMDVSTQLQATCNVHKSAFNDFWSGHLNFQIEHHL
                |||||:|||:|||:|||:|||:|||:|||:|||:|||:|||:|||
2535            FLESHWFVWWTQMNHIVMEIDQEAYRDWFSSQLTATCNVEQSFFNDFWSGHLNFQIEHHL
               180        190        200        210        220        230

pRAE-7.pep      240         250         260         270         280
                  FPTMPRHNYHKVAPLVQSLCAKHGIEYQSKPLLSAFADIHSLKESGQLWLDAYLHQX
                |||||:|||:|||:|||:|||:|||:|||:|||:|||:|||:|||
2535            FPTMPRLNLKLIAPLVKSLCAKHGIEYQEKPLLRALLDIRSLKSGKLWLDAYLHKXSH
               240        250        260        270        280        290

2535            SPRTIVGKGCRWGEGQRNDGLLFXGVSERLVYALLTDPMLDLSPFLSFSSSLPHSTLP
               300        310        320        330        340        350
```

Figure 18

20/40

## FastA Match of the Gene in pRAE-7 and contig 38

SCORES      Initl:    965    Initn:    965    Opt:    968  
 Smith-Waterman score: 968;    97.0% identity in 133 aa overlap

```

                                10      20      30      39
pRAE-7.pep                      LLEFVSI GGI PAVQA QAGWLQ HDFGHL SVFSTSKWNHLL
                                : ||||| ||||| ||||| ||||| |||||
38      LHI LLLDGA AWTL WVF GTSFL PFL LCAV LLSAVQA QAGWLQ HDFGHL SVFSTSKWNHLL
                                130      140      150      160      170      180

                                40      50      60      70      80      90      99
pRAE-7.pep      HHFVIGH LKGAPAS WWNHMFQ HHAKPNC FRKDPD INMHPFF FALGKILS VELGKQKKKY
                                ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
38      HHFVIGH LKGAPAS WWNHMFQ HHAKPNC FRKDPD INMHPFF FALGKILS VELGKQKKKY
                                190      200      210      220      230      240

                                100      110      120      130      140      150      159
pRAE-7.pep      MPYNHQH KYFFLIG PPA LPL YFQWY IFYFVI QKKWV DLA WMITFY VRFFLT YVPLLGL
                                ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
38      MPYNHQH KYFFLIG PPA LPL YFQWY IFYFVI QKKWV DLA WISKQEYDEAGLPLSTANA
                                250      260      270      280      290      300

                                160      170      180      190      200      210      219
pRAE-7.pep      KAFLGLFF IVRFL ESNW FVWVTQ MNH IPMH IDH DRNMD WSTQLQATCNVHKS AFNDWFS
38      SK
  
```

Figure 19

21/40

### FastA Match of the N-terminus of Clone A-1 and Human Cytochrome b5

A-1.pdt  
SW:CYB5 HUMAN

```
ID CYB5_HUMAN .STANDARD; PRT; 133 AA.
AC P00167;
DT 21-JUL-1986 (REL. 01, CREATED)
DT 01-NOV-1988 (REL. 09, LAST SEQUENCE UPDATE)
DT 01-FEB-1996 (REL. 33, LAST ANNOTATION UPDATE)
DE CYTOCHROME B5. . . . .
```

SCORES Init1: 127 Initn: 127 Opt: 183 z-score: 226.9 E(): 5.4e-06  
Smith-Waterman score: 183; 32.2% identity in 90 aa overlap

```

      530          540          550          560          570          580
A-1.pdt XLDLPTNMEXRKAAAELXAETAACGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISE
              :|:| |:|: ::| :||:::|||::::
CYB5_HUMAN AEQSDSEAVKYTLEETQKHNHSKSTWLLHHKVYDLTK
                        10           20           30


      590          600          610          620          630          640
A-1.pdt FTRRHGGSRVISHLAGQDATDPFVAFHINKGLVKKYMN-SLLIGELSFEQPSFEPTKNK
|::||| |:|: |||||:| :::: |:|: ::||| |:|: |
CYB5_HUMAN FLEEHPGGEEVLREQAGGDATENFE--DVGHSTDAREMSKTFIIGELHPDD---RPKLNK
40             50             60             70             80             90


      650          660          670          680          690          700
A-1.pdt ELTDEFRELRAIVEQRFPVXFLCTCGAGFFSLEVPLPDSNKXFSWISRPDANKGKRP
PEETILITTIDSSSWINMWIPASAVAVALMYRLYMAED
100            110            120            130

```

Figure 20



22/40

## FastA Match of 5' Sequence of Clone A-1 and ac004228

LOCUS . AC004228 170743 bp DNA HTG 26-FEB-1998  
 DEFINITION \*\*\* SEQUENCING IN PROGRESS \*\*\* Homo sapiens Chromosome 11q12 pac  
 pDJ519o3; HTGS phase 1, 18 unordered pieces.  
 ACCESSION AC004228  
 NID g2911733  
 KEYWORDS HTG; HTGS\_PHASE1. . . .

SCORES Init1: 913 Initn: 1123 Opt: 916  
 94.6% identity in 203 bp overlap

A-1	389	379	369	359	349	339	330
	CCCGACCAATATGATGGAATAAAGGAAAGCGGCCGCTGAATTATAGGCCGCCGAGACCGC						
ac004228	CCCGGCGCGCGCGTCCGACGGCCAGCTATGGCCCCGACCCGGTGGCCGCCGAGACCGC						
	60090	60100	60110	60120	60130	60140	
A-1	329	319	309	299	289	279	270
	GGCTCAGGGACCTACCCGCGTTACTTCACATGGGACGAGGTGGCCCAGCGCTCAGGGTG						
ac004228	GGCTCAGGGACCTACCCGCGTACTTCACCTGGGACGAGGTGGCCCAGCGCTCAGGGTG						
	60150	60160	60170	60180	60190	60200	
A-1	269	259	249	239	229	219	210
	CGAGGAGCGGTGGCTTGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTACCCGCCCG						
ac004228	CGAGGAGCGGTGGCTAGTGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTACCCGCCCG						
	60210	60220	60230	60240	60250	60260	
A-1	209	199	189	179	169	159	150
	GCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGATCCCTT						
ac004228	GCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGTGAGCGC						
	60270	60280	60290	60300	60310	60320	
A-1	149	139	129	119	109	99	90
	CGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAATATATGAACCTCTCTCCTGATTGG						
ac004228	AGCCAGGCGGGGACAGGAGAGGGCGGGACCGGAGGCTAGTGCAGGGGAGACAGAGTT						
	60330	60340	60350	60360	60370	60380	

Figure 21

23/40

## FastA Match of 5' Sequence of Clone 3-5 and ac004228

SCORES Initl: 1365 Initn: 2510 Opt: 1377  
98.6% identity in 285 bp overlap

	20	30	40	50	60	70
3-5	AATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGCAGGTCCGGACCTGCCAACGTGA					
ac004228	CCCCGCCCCACACGCCGCATCACTTACAGGGCCCGGGCTG-CCGGACCTGCCAACGTGA					
	61710	61720	61730	61740	61750	61760
	80	90	100	110	120	130
3-5	ATCTTATCGCCATGGACCTTACCTTGCACAACCCAAAGTAGCTGCCTTGGGGCAGGGGGT					
ac004228	ATCTTATCGCCATGGACCTTACCTTGCACAACCCAAAGTAGCTGCCTTGGGGCAGGGGGT					
	61770	61780	61790	61800	61810	61820
	140	150	160	170	180	190
3-5	GGCCAGAGTGCTTAGGGAAATGTGGAGCCCTACCCAGAACAACGGTGGAGGGAAAGGGAA					
ac004228	GGCCAGAGTGCTTAGGGAAATGTGGAGCCCTACCCAGAACAACGGTGGAGGGAAAGGGAA					
	61830	61840	61850	61860	61870	61880
	200	210	220	230	240	250
3-5	GAAACGCAGAAGTGCCCCAGTTCGGACGTAGGGAAAGTCTTCCTCTTCGTGGTTTTGGAG					
ac004228	GAAACGCAGAAGTGCCCCAGTTCGGACGTAGGGAAAGTCTTCCTCTTCGTGGTTTTGGAG					
	61890	61900	61910	61920	61930	61940
	260	270	280	290	300	310
3-5	AACCCCTAGCTAAGAGAGGAAAGGGACTTATTGAAAGACCCGCAAGAAGGGACGGAAGTCT					
ac004228	AACCCCTAGCTAAGAGAGGAAAGGGACTTATTGAAAGACCCGCAAGAAGGGACGGAAGTCT					
	61950	61960	61970	61980	61990	62000
	320	330	340	350	360	370
3-5	CATAGCCCTGAGAGGATCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTAAGAAGT					
ac004228	CATAGCCCTGAGAGGTGAAGCCAGCTGGAGTTGATGGGTGGAATGGGGACCTAGAGAACT					
	62010	62020	62030	62040	62050	62060

Figure 22

24/40

## FastA Match of 5' Sequence of Clone A-10 and ac004228

SCORES Init1: 931 Initn: 1309 Opt: 934  
 97.0% identity in 200 bp overlap

	30	40	50	60	70	80	89
A-10	TATAGGGCTCGAGCGGCCCGGGCAGGTGCCCGGAGGCGCCTGATCATACCTGTGTC						
ac004228	CGAGCCAAACACCGACTAATTCGGAGGAAAGCCCGGAGGCGCCTGATCATACCTGTGTC						
	60400	60410	60420	60430	60440	60450	
	90	100	110	120	130	140	149
A-10	CGGTGATTGGGTGTCCTGCGGATGCGGGATGAAAAGGCGGAGAGAGGCCCTGGAAAAGTG						
ac004228	CGGTGATTGGGTGTCCTGCGGATGCGGGATGAAAAGGCGGAGAGAGGCCCTGGAGAAGTG						
	60460	60470	60480	60490	60500	60510	
	150	160	170	180	190	200	209
A-10	GAGTCTGGGGAGTGGGGATGGAGGCCAACAACACGCACACACAAACAAAGGGTCCCGCCT						
ac004228	GAGTCTGGGGAGTGGGGATGGAGGCCAACAACACGCACACACAAACAAAGGGTCCCGCCT						
	60520	60530	60540	60550	60560	60570	
	210	220	230	240	250	260	269
A-10	CCCTGCCGTGCATTCCATCTGCAGCCCCGAGCCTCAGGATCCCTTTGTGGCCTTCCACAT						
ac004228	CCCTGCCGTGCATTCCATCTGCAGCCCCGAGCCTCAGG-TCTCTGGGCGGGACAGAACC						
	60580	60590	60600	60610	60620	60630	

Figure 23

25/40

## FastA Match of 5' Sequence of Clone A-16 and ac004228

SCORES Initl: 985 Initn: 1488 Opt: 997  
 98.1% identity in 209 bp overlap

	40	50	60	70	80	90
A-16	CGAGCGGCCGCCCCGGCAGGCTCAGAAATTCAGCGGCCGCTGAAGCCGCTCTGGACCTAG					
ac004228	AGGGAGTCACATCCTGTCTCGGATGGCTAGGAGAGGCAGC-GCAGCCGCGCTCTGGACCTAG					
	60720	60730	60740	60750	60760	
	100	110	120	130	140	150
A-16	GTGCCGCTCTCCACTCGCCAGCAGGAGCGGAGAGGAGCAGGAAAGGAGCCCATTCCTCGA					
ac004228	GTGCCGCTCTCCACTCGCCAGCAGGAGCGGAGAGGAGCAGGAAAGGAGCCCATTCCTCGA					
	60770	60780	60790	60800	60810	60820
	160	170	180	190	200	210
A-16	GGATGGGGCTGAAACGGGAAGCTTGGGGAGACCGCTGCCTTGGGGACCCCTGCGTCGTGT					
ac004228	GGATGGGGCTGAAACGGGAAGCTTGGGGAGACCGCTGCCTTGGGGACCCCTGCGTCGTGT					
	60830	60840	60850	60860	60870	60880
	220	230	240	250	260	270
A-16	GAAGACTGGAGGACGCGGAAGGGACAGCGCTGGCCGGGAGGGCAAGCGGCCGCTGGCGA					
ac004228	GAAGACTGGAGGACGCGGAAGGGACAGCGCTGGCCGGGAGGGCAAGCGGCCGCTGGCGT					
	60890	60900	60910	60920	60930	60940
	280	290	300	310	320	330
A-16	TCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAACCTCTCTCCT					
ac004228	ACATAAGGGATTGGGAATGGCATACTTAGCGAGGACCCCAAGAGCTGTTCTCGAATCG					
	60950	60960	60970	60980	60990	61000

Figure 24

26/40

## FastA Match of 5' Sequence of Clone A-19 and ac004228

SCORES Initl: 1227 Initn: 1409 Opt: 1532  
94.0% identity in 349 bp overlap

```

      60      70      80      90      100     110
A-19      TTATTCCCTTATTGTCCCTGCCCATGTCCTGCTGATTGGTCCATTTTACCTCTAGCTAG
ac004228   ATAGAGCACTGATTGGTCCATTTTACAGGGTGCTGATTGGTCCATTTTACCTCTAGCTAG
      63250   63260   63270   63280   63290   63300

      120     130     140     150     160     170
A-19      CTAAAGAGCACGGATTGGTGCATTTTGCAAACCTCTGGCTACAGAGGGGTTCTCCAGGTC
ac004228   CTAAAGAGCACGGATTGGTGCATTTTACAAACCTCTAGCTACAGAAAAGTTCTCCAAGTC
      63310   63320   63330   63340   63350   63360

      180     190     200     210     220     230
A-19      TGCACTCGACCCAGGAAGTCCATCTGGCTTCACCTCTCACTTCAACTTGGGTACAGCCTT
ac004228   TGCACTCGACCCAGGAAGTCCATCTGGCTTCACCTCTCACTTCAACTTGGGTACAGCCTT
      63370   63380   63390   63400   63410   63420

      240     250     260     270     280     290
A-19      CTGGCGGGCAGGAAGATGGCCTTTGGTGCGAACACTGCCGGAGTCCAGGGGGCTGGCTCC
ac004228   CTGGCGGGCAGGAGGATGGCCTTTGGTGCGAACACTGCCGGAGTCCAGGGGGCTGGCTCC
      63430   63440   63450   63460   63470   63480

      300     310     320     330     340
A-19      CTCACCTTTCATCTTCTCCCGGCACTTGCAGGATCCCTTTGTGGCC
ac004228   CTCACCTTTCATCTTCTCCCGGCACTTGCAGGATCCCTTTGTGGCC
      63490   63500   63510   63520   63530
```

Figure 25

27/40

## Partial Sequence of ac004228

59751 ACTAGAACCG CTGTTCTTAC CGCGGCGCCC CCTGGGAGCC AACGCCCGCA  
 59801 TGCCCGCCTG ACGTCAGGAA GTCGAATCCG GCGGCGACGC TTTTAGGGAG  
 59851 CCCGCGAGGG GGCGCGTGTT GGCAGCCCAG CTGTGAGTTG CCCAAGACCC  
 59901 ACCGGGGGAC GGGATCTCGC TCCCCGCGCC ACGAGGCTCG GCCAATGGGA Possible start  
 59951 ACGCGCGCTG CGAGGCCCGC CGGTCTGCCC TGCGGTGCTG AAAACCCGGC  
 60001 GCGCAGGCGG CTGGCTCTGG GCGCGCGCCA GCAAATCCAC TCCTGGAGCC  
 60051 CGCGGACCCC GAGCAGCGGC CTGACAGCCC CTGCTGGCCC GGCGCGCGGC  
 60101 GTCGCCAGGC CAGCTATGGC CCCCAGACCG GTGGCCGCGG AGACCGCGGC  
 60151 TCAGGGACCT ACCCGCGGCT ACTTCACCTG GGACGAGGTG GCCCAGCGCT Clone A-1  
 60201 CAGGGTCCGA GGAGCGGTGG CTAGTGATCG ACCGTAAGGT GTACAAGATC  
 60251 AGCGAGTCA CCCGCGGCA TCCAGGGGGC TCCCGGGTCA TCAGCCACTA  
 60301 CGCCGGGCGG GATGCCGCGG TGAGCGCAGC CAGGCGGGGG CACAGGAGAG  
 60351 GCGGGGACCG GAGGCTGAGT GCAGGGGAGA CAGAGTTACG CACTCCGAGC  
 60401 CAAACACCGA CTAATTCGGA GGAAAGCCCG GAGGCGCCTG ATCATACCTG  
 60451 TTGCCCCGTG ATTGGGTGTC CTGCGGATGC GGGATGAAAA GCGGGGAGAG Clone A-10  
 60501 AGGCGTGGAG AAGTGGATTC TGGGGAGTGG GGATGGAGGC CAACAACAGC  
 60551 CACAGATGAA GAAAGGCTCC TGCCTCCCTG CCGTGCAATC CATCTGCAGC  
 60601 CGGAGGCTG AGGTCTCTGG GCGGGGACAG AACCCCGAGC TGGGTAGGCT  
 60651 AGGAGGGAGG AGAGCAAGGA TGCAGGCCGC CTGGGGAGGG AGGGGGTCAG  
 60701 TGGCTAGGGG AGGGAGTCAC ATCCTGTCTC GATGGCTAGG AGAGGCAGCG  
 60751 GAGCGGCTG TGGACCTAGG TGGCGGTCTC CACTCGCCAG CAGGAGCGGA Clone B-1  
 60801 GAGCGGCTG GAAAGGCTCC CATCTCTGAG GATGGGGCTG AAACGGGAAG  
 60851 CTTGGGCTG GCGCTGCTT TGGGACCCCT GCGTCGTGTG AAGACTGGAG  
 60901 GACGCGGAG GACAGGCTT GCGCGGGGAG GGCAAGCGGC CGCTGGCGTA  
 60951 CATAGGATAT TGGGAATGGC ATACACTTAG CGAGGACCCC CAGAGCTGTT  
 61001 CTCGAATGAG TGGGAGGCTC ACTGAGCCGC AGGCCAGCGA GTCCTTCAGC

Figure 26a

28/40

61051 TATTCGCGG AGCGGACCGC TGTTACGCT CTGGGCGGT AGGCCCTTCG  
 61101 CGGGGTCTTG TCCCTTCTTC CCTTGGTCTC ACTGCGGGT CGGCGCGGC  
 61151 CCCAGCCCCA GGCTGCTGC TTCCCTTTCT AGACCACAGC CCTCAGAGCT  
 61201 AAGGCCCGG CGCTCTCTG CTGGGTGGA GTCCTGGGA CTCAGTCTA  
 61251 GGGACTCGAA AGTCGGGCG TTCCCTTCAC CGCGTTCCC CCTTGGCGGC  
 61301 CAGAATGGCG TCCCCTCCC TTGCATCCCC CTCTGATCCC GTGCCCTGCA  
 61351 GCGTGATGCC CTCCACTGTC CCTATCCACT ACCCTGGCGT CCCAGAGTGT  
 61401 GCCGCGGGTC ACCAGGTTC CATAACGTCG CAGCAGAGCT TAGACGCTGC  
 61451 GGGCGAAGA CCCGCCCCAC CCTCTGACGC GACCAGCCTA GTGGGCGAGG  
 61501 CCAGAGCTTG CGCGGTCAA CCAGAGTGAC CACTCGGGAG CCCTGACTGC  
 61551 GGCCAAGGGC GCAGGCGTGT CCCGGCGCAT GCGCAGACGA AACAGGCACC  
 61601 AACGCTGGAG CTTCCCGCAG TGTGATTTGG GGCCGGGAT GCCGCGCGG  
 61651 GGACGGCGAT TGGTCCGTAT GTGTGGTGCC ACCGGCCGCC GCTCCGCCCC  
 61701 GGCCCCCGCC CCACACGCCG CATCACTTAC AGGGCCCGGG GCTCCGGAC  
 61751 CTGCCAACGT GAATCTTATC GCCATGGACC TTACCTTGCA CAACCCAAAG  
 61801 TAGCTGCCCTT GGGCAGGGG GTGGCCAGAG TGCTTAGGA AATGTGAGC Clone 3-5  
 61851 CCTACCCAGA ACAACGGTGG AGGGAABGGG AAGAAACGCA GAAGTSCCCC  
 61901 AGTTCGGAGG TAGGGAAGTC TTCTCTTCG TGTTTTTTGG AGAACCTAG  
 61951 CTAAGAGAGG AAAGGGACTT ATTGAAAGAC CCGCAAGAAG GACGGAAGT  
 62001 CTCATAGCCC TGAGAGGTGA AGCCAGCTGG AGTTGATGGG TCGAATGGGG  
 62051 ACCTAGAGAA CTTTTCTGTA TCTAGAGGTT TGTAAAATGC ACCAATCAGT  
 62101 GCTCTGTAAA AACGCACCAA TTGGCGCTCT GTAGCTAGCT AGAGGTTTGT  
 62151 AAAATGAGCC AATCAGCAGG ACGTGGGCAG GGACAACTAA GACAATAAAA  
 62201 GCTGGCCACC CCAGCCAGCT GCTGCAACCC GCTCCAGTTC CTTACAGGC  
 62251 TGTGGAAGCA TTGTTCTTTT GCTCGTCACA CTAAACCTTG TTGCTGCTCA  
 62301 TTCTTTGGGT CTGCAAAGAG TGTATTCTCT TTAAGAGCTA TAACAGCGGG  
 62351 AAGGTCCACG GCTCCATTCT TGAAGTCAGT GAGACCATAC TTCCCGAAG  
 62401 GAACCAACGC CCGACACAGC CCCACCCATC TCTCTGTTT CTCACCTATA

Figure 26b

29/40

62451 CTGAAATTCT TGGGCAAAG CTGTCTGTGG ACACACCCAG GGGAAAGGCC  
 62501 AGCCCAAGCA GGTGTTTCTT AGTGGTTCCC CTCAGCCAAT GCTTCCCATT  
 62551 CCTTGATGCA TCCTTCTAAC TAGAGCAGAT GCTCGGTGAT CTTAAACTGT  
 62601 GGACACCTGG GAGCACCCTC AAAAGGCAGC TGGGCCTAGG GAGATGGCCT  
 62651 GTGCTTCTGT GTCAGGAGTT GGTTCCTTCA GGTGGGCTTG TGGTCTCGCT  
 62701 GACGTCAAGA ATGAAGCCAT GAACCTTCGC GGTGAGTGT ACAGCTCTTA  
 62751 CAGGTGGCGT GGACCCAAAG AGTGAGCAGC AGCAAGATTT ATTGTGAAGA  
 62801 GCAAAGAACA AAGCTTCCAC AGCGTGGAAG GGTACCCGAG CAGGTGCGCG  
 62851 CTGCTGGACG TTGGGGGGTG TGAGGGGGAG CAGCCTTTTT TTTTCTTTTT  
 62901 TTTTGGAGAC GGAGTCTCCC TGTCGCCCAG GCTGGAGTGC AGTGGCGCGA  
 62951 TCTCGGCTCA CTGCAGGCTC CGCCCCCCCC CCGGGGTTC ACGCATTCCT  
 63001 CTGCCTCAGC CTCCCGAGTA GCTGGGACTA CAGGCGCCCG CTACCTCGCC  
 63051 CGGCTAATTT TTTGTATTTT TAGTAGAGAC GGGGTTTCAC TGTGTTAGCC  
 63101 AGGATGGTCT CGATCTCCTG ACCTCGTGAT CCACCCGCTT TGGCCTCCCA  
 63151 AAGTGCTGGG ATTACAGGCG TGAGCCACCG CGCCCGGCCG GGAGCAGCTT  
 63201 TTATTCCTT ATTGTCCCT GCCCATGTCC TGCTGATTG TCCATTTTAT  
 63251 AGAGCACTGA TTGGTCCATT TTACAGGGTG CTGATTGGTC CATTTTACCT  
 63301 CTAGCTAGCT AAAGAGCAGC GATTGGTGCA TTTTACAAAC CTCTAGCTAC  
 63351 AGAAAGTTC TCCAAGTCTG CACTCGACCC AGGAAGTCCA TCTGGCTTCA  
 63401 CCTCTCACTT CAACTTGGGT ACAGCCTTCT GCGGGGAGG AGGATGGCCT  
 63451 TTGGTGCGAA CACTGCGGGA GTCCAGGGGG CTGGCTCCCT CACCTTTCAT Clone B-19  
 63501 CTCTCCCGG CACTTGCAGG ATCCCTTTGT GGCCTTCCAC ATCAACAAGG  
 63551 GCCTTGTGAA GAAGTATATG AACTCTCTCC TGATTGGAGA ACTGTCTCCA  
 63601 GAGCAGCCCA GCTTTGAGCC CACCAAGAA GTAAGACCCT GTGTTTGCTA  
 63651 TGTSCAACT ATTGGTTGTT GAGGGGGACA GAGAGGGGGT GGAAGGAGAG  
 63701 TCTAGATGGA ATCAGAGTCA TAGTAATCAC AGTCAGTAGT AGCTCTGGGG  
 63751 AATTTGAGG TCCCTGCTTC TCTTGCAATG TCATGAGGTC ACAGGCCCAA

Figure 26c



30/40

63801 GGGAGCATGG CTTTGCAACC TATGGCTCCC CCAAGGCTGC CACTACCATG  
63851 GCTGCCATCA TTGTTATCAT CATTGTTATC ATATGAGCAC TTACTATGCA  
63901 CCAAGCATAA ACTCATAACT CTTACACATT TACAGATGAG ATAACAGGCT  
63951 CAGGGAGGTT AAGCAACACA GCCAAGGATC ACACAGTTAG TAAATGGCAG  
64001 AGCAAGGACT TAGTCCCCTG AACTCTTAGG CACTATCCCA TGGCACCTCC  
64051 TCCTGTCATC CTCATTGTCG TGGTATCTTT GCCTAGGACT GTGGACTTCC  
64101 CACAGCTACC TCAGTGGGAG GTCCTTGAGC CTGAGAGGGC CCTTGTCTCC  
64151 AGTAGCATTG GGGTGCAGAT GAGAAGAATA ACAGCTCCTC TTCCTCTTCT  
64201 GCAGAAAGAG CTGACAGATG AGTTCCGGGA GCTGCGGGCC ACAGTGGAGC  
64251 GGATGGGGCT CATGAAGGCC AACCATGTCT TCTTCCTGCT GTACCTGCTG  
64301 CACATCTTGC TGCTGGATGG TGCAGCCTGG CTCACCTTTT GGGTCTTTGG  
64351 GACGTCCCTT TTGCCCTTCC TCCTCTGTGC GGTGCTGCTC AGTGCAGTTC  
64401 AGGTGAGAGC CTTTGGCTTG TCAAGTGCAC AGCAATGCTC AGCATCCCTG

Figure 26d

31/40

### FastA Match of Human $\Delta 5$ -desaturase and Contig 3381584

SCORES Init1: 4480 Initn: 4480 Cpt: 4431  
99.9% identity in 898 bp overlap

human $\Delta 5$				10	20	30
				ATGGCCCCGACCCGGTGGCCGCCGAGACC		
3381584	GGCCCCGGCGCGGGCGTCGCCAGGCCAGCTATGGCCCCGACCCGGTGGCCGCCGAGACC					
	80	90	100	110	120	130
human $\Delta 5$		40	50	60	70	80
	GCGGCTCAGGGACCTACCCCGCGCTACTTCACCTGGGACGAGGTGGCCGAGCGCTCAGGG					
3381584	GCGGCTCAGGGACCTACCCCGCGCTACTTCACCTGGGACGAGGTGGCCGAGCGCTCAGGG					
	140	150	160	170	180	190
human $\Delta 5$		100	110	120	130	140
	TGCGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTCACCCGC					
3381584	TGCGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTCACCCGC					
	200	210	220	230	240	250
human $\Delta 5$		160	170	180	190	200
	CGGCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGATCCC					
3381584	CGGCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGATCCC					
	260	270	280	290	300	310
human $\Delta 5$		220	230	240	250	260
	TTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAAGTCTCTCTGATT					
3381584	TTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAAGTCTCTCTGATT					
	320	330	340	350	360	370
human $\Delta 5$		280	290	300	310	320
	GGAGAACTGTCTCCAGAGCAGCCAGCTTTGAGCCCAACCAAGAAATAAGAGCTGACAGAT					
3381584	GGAGAACTGTCTCCAGAGCAGCCAGCTTTGAGCCCAACCAAGAAATAAGAGCTGACAGAT					
	380	390	400	410	420	430
human $\Delta 5$		340	350	360	370	380
	TAGTTCCGGGAGCTGCGGGCCACAGTGGAGCGGATGGGGCTCATGAAGGCCAACCATGTC					
3381584	TAGTTCCGGGAGCTGCGGGCCACAGTGGAGCGGATGGGGCTCATGAAGGCCAACCATGTC					
	440	450	460	470	480	490
human $\Delta 5$		400	410	420	430	440
	TTCTTCTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCCTT					
3381584	TTCTTCTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCCTT					
	500	510	520	530	540	550

Figure 27a

32/40

	460	470	480	490	500	510
human Δ5	TGGGTCTTTGGGACGTCCCTTTTGGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCCAGTT					
3381584	TGGGTCTTTGGGACGTCCCTTTTGGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCCAGTT					
	560	570	580	590	600	610
human Δ5	520	530	540	550	560	570
	CAGGCCCAGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCA					
3381584	CAGGCCCAGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCA					
	620	630	640	650	660	670
human Δ5	580	590	600	610	620	630
	AAGTGAACCATCTGCTACATCATTTTGTGATTGGCCACCTGAAGGGGGCCCCGCCAGT					
3381584	AAGTGAACCATCTGCTACATCATTTTGTGATTGGCCACCTGAAGGGGGCCCCGCCAGT					
	680	690	700	710	720	730
human Δ5	640	650	660	670	680	690
	TGGTGAACCATGCACTTCCAGCACCATGCCAAGCCCAACTGCTTCCGCAAAGACCCA					
3381584	TGGTGAACCATGCACTTCCAGCACCATGCCAAGCCCAACTGCTTCCGCAAAGACCCA					
	740	750	760	770	780	790
human Δ5	700	710	720	730	740	750
	GACATCAACATGCATCCCTTCTTCTTTGCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGG					
3381584	GACATCAACATGCATCCCTTCTTCTTTGCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGG					
	800	810	820	830	840	850
human Δ5	760	770	780	790	800	810
	AAACAGAAGAAAAATATATGCCGTACAACCACCAGCACAAATACTTCTTCCTAATTGGG					
3381584	AAACAGAAGAAAAATATATGCCGTACAACCACCAGCACAAATACTTCTTCCTAATTGGG					
	860	870	880	890	900	910
human Δ5	820	830	840	850	860	870
	CCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTCTATTTTGTATCCAGCGA					
3381584	CCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTCTATTTTGTATCCAGCGA					
	920	930	940	950	960	970
human Δ5	880	890	900	910	920	930
	AAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCCTCACTTAT					
3381584	AAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCCTCACTTAT					
	980	990	1000	1010	1020	1030

Figure 27b

33/40

### FastA Match of Human $\Delta 5$ -desaturase and Contig 2153526

SCORES Initl: 1892 Initn: 1892 Opt: 2161  
98.6% identity in 443 bp overlap

	870	880	890	900	910	920
human $\Delta 5$	TCCAGCGAAAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCC					
2153526				GAATKMTTACCTTCTACGTCCGCTTCTTCC		
				10	20	30
	930	940	950	960	970	980
human $\Delta 5$	TCACTTATGTGCCACTATTGGGGCTGAAAGCCTTCCTGGGCCTTTTCTTCATAGTCAGGT					
2153526	TCACTTATGTGCCACTATTGGGGCTGAAAG-CTTCCTGGGCCTTTTCTTCATAGTCAGGT					
	40	50	60	70	80	
	990	1000	1010	1020	1030	1040
human $\Delta 5$	TCCTGGAAAGCAACTGGTTTGTGTGGGTGACACAGATGAACCATATTCCCATGCACATTG					
2153526	TCCTGGAAAGCAACTGGTTTGTGTGGGTGACACAGATGAACCATATTCCCATGCACATTG					
	90	100	110	120	130	140
	1050	1060	1070	1080	1090	1100
human $\Delta 5$	ATCATGACCGGAACATGGACTGGGTTCACCCAGCTCCTGGCCACATGCAATGTCCACA					
2153526	ATCATGACCGGAACATGGACTGGGTTCACCCAGCTCCAGGCCACATGCAATGTCCACA					
	150	160	170	180	190	200
	1110	1120	1130	1140	1150	1160
human $\Delta 5$	AGTCTGCCTTCAATGACTGGTTTCAGTGGACACCTCAACTTCCAGATTGAGCACCATCTTT					
2153526	AGTCTGCCTTCAATGACTGGTTTCAGTGGACACCTCAACTTCCAGATTGAGCACCATCTTT					
	210	220	230	240	250	260
	1170	1180	1190	1200	1210	1220
human $\Delta 5$	TTCCACGATGCCTCGACACAATTACCACAAAGTGGCTCCCTGGTGCAGTCTCTGTGTG					
2153526	TTCCACGATGCCTCGACACAATTACCACAAAGTGGCTCCCTGGTGCAGTCTCTGTGTG					
	270	280	290	300	310	320
	1230	1240	1250	1260	1270	1280
human $\Delta 5$	CCAAGCGTGGCATAGAGTACCAGTCCAAGCCCTGCTGTCAGCCTTCGCCGACATCATCC					
2153526	CCAAGCATGGCATAGAGTACCAGTCCAAGCCCTGCTGTCAGCCTTCGCCGACATCATCC					
	330	340	350	360	370	380
	1290	1300	1310	1320	1330	
human $\Delta 5$	ACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCCTATCTTCACCAATAA					
2153526	ACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCCTATCTTCACCAATAA					
	390	400	410	420	430	440

Figure 28

34/40

### FastA Match of Human $\Delta 5$ -desaturase and Contig 253538a

SCORES Initl: 1479 Initn: 2483 Opt: 2489  
Smith-Waterman score: 2489; 81.3% identity in 434 aa overlap

human $\Delta 5$	10	20	30	40	50	60
253538a	MAPDPVAAETAAGGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVIS					
		10	20	30	40	
human $\Delta 5$	70	80	90	100	110	120
253538a	HYAGQDATDPFVAFHINKGLVKKYMNSLLIGELSP EQPSFEPTKNKELTDEFREL RATVE					
	50	60	70	80	90	100
human $\Delta 5$	130	140	150	160	170	180
253538a	RMGLMKANHVFLLYLLHILLDGAAWLTWVFGTSFLPFLCAVLLSAVQAQAGWLQHD					
	110	120	130	140	150	160
human $\Delta 5$	190	200	210	220	230	239
253538a	FGHLSVSTSKWNHLLHHFVIGHLKGAPASWWNHMFQHHAKPNCFRKDPDINM-HPFFF					
	170	180	190	200	210	220
human $\Delta 5$	240	250	260	270	280	290
253538a	ALGKILSVELGKQKKKYPYNHQQHYFFLIGPPALLPLYFQWYIFVVIQRKKWDLAWM					
	230	240	250	260	270	280
human $\Delta 5$	300	310	320	330	340	350
253538a	ITFYVRFFLTIVPPLG-LKAFGLFFIVRFLESNWFVWVTQMNHHPMHIDHNRNDWVST					
	290	300	310	320	330	340
human $\Delta 5$	360	370	380	390	400	410
253538a	QLLATCNVHKSAFNDWFSGHLNFQIEHHLFFTPMPPHNTKVAPLVQSLCAKRGIE/QSKP					
	350	360	370	380	390	400
human $\Delta 5$	420	430	440			
253538a	LLSAFADIIHSLKESGQLWLDAYLHQX					
	410	420	430	440	450	460
	LLRALLDIIRSLKXSGKLWLDAYLHFKXSHSPRDTVGKGCPRWGGQRFNDEGLLFXGVSERLV					

Figure 29

35/40

FastA Match of Human  $\Delta 5$ -desaturase and Contig 38

SCORES Initl: 2024 Initn: 2024 Opt: 2026  
 Smith-Waterman score: 2026; 99.3% identity in 287 aa overlap

```

      10      20      30      40      50      60
human  $\Delta 5$  MAPDPVAAETAAQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVIS
38      QGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVIS
              10      20      30      40

      70      80      90     100     110     120
human  $\Delta 5$  HYAGQDATDPFVAFHINKGLVKYMNSSLIGELSPEQPSFEPTKNKELTDEFRELRAIVE
38      HYAGQDATDPFVAFHINKGLVKYMNSSLIGELSPEQPSFEPTKNKELTDEFRELRAIVE
      50      60      70      80      90     100

      130     140     150     160     170     180
human  $\Delta 5$  RMGLMKANHVFLLYLLHILLLDGAAWLTWVFGTSFLPFLLCVLLSAVQAQAGWLQHD
38      RMGLMKANHVFLLYLLHILLLDGAAWLTWVFGTSFLPFLLCVLLSAVQAQAGWLQHD
      110     120     130     140     150     160

      190     200     210     220     230     240
human  $\Delta 5$  FGHLSVFSSTSKWNHLLHHFVIGHLKGAPASWNNMHFQHHAKPNCFRKDPDINMHPFFFA
38      FGHLSVFSSTSKWNHLLHHFVIGHLKGAPASWNNMHFQHHAKPNCFRKDPDINMHPFFFA
      170     180     190     200     210     220

      250     260     270     280     290     300
human  $\Delta 5$  LGKILSVELGKQKKKYPYNHGHXYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWMI
38      LGKILSVELGKQKKKYPYNHGHXYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWIS
      230     240     250     260     270     280

      310     320     330     340     350     360
human  $\Delta 5$  TFYVRFFLTIVPLLGLKAFLGLFFIVRFLESNWFVWVTQMNHIFMHIDHNRNMDWVSTQL
38      KQEYDEAGLPLSTANASKRDLPRATSPGTRWPSAQGARSGGXXSTVRCITTSASSPAGIQG
      290     300     310     320     330     340

```

Figure 30

36/40

### FastA Match of $\Delta 6$ -Desaturase (Ma524) and Human $\Delta 5$ -Desaturase

SCORES Initl: 280 Initn: 601 Opt: 303  
Smith-Waterman score: 697; 30.5% identity in 455 aa overlap

```

      10      20      30      40      50
human  $\Delta 5$  MAPDPVAAETAAGQPTPRYFTWDEV---AQRSGCEER---WLVIDRKVYNISEFTRRH
      | : | : | : | : | : | : | : | : | : | : | : | : | : | : | : | : |
Ma524.pep MAAAPSVRTTFAEVLNAEALNEGKKDAEAPFLMIIDNKVYDVREFVDPH
      10      20      30      40      50

      60      70      80      90     100     110
human  $\Delta 5$  PGGSRVISHYAGQDATDPFVAFHINKGLVKYMNLSLLIGELSPEQPSFEPTKKNELTDEF
      | | | : : | : : | : | : | : : : : : : : : : | : | : : |
Ma524.pep PGGSVILTH-VGKDGTVDFDTFHPEAAW--ETLANFYVGDIIDE---SDRDIKNDDFAAEV
      60      70      80      90     100

      120     130     140     150     160     170
human  $\Delta 5$  RELRATVERMGLMKANHVFLLYLLHILLLDGAAWLTLWVFG-TSFLPFLLCVLLSAVQ
      | : | : : | : : : : : : : : : : : | : | : | : | : | : |
Ma524.pep RKLRTLQSLGYDSSKAYYAFKVSFNLCTWGLSTVIVAKWGQSTLANVLSAALLGLFW
      110     120     130     140     150     160

      180     190     200     210     220     230
human  $\Delta 5$  AQAGWLQHDFGHLVSFSTSKWNHLLHFFVIGHLKGAPASWWNHMFQHHAKPNCFRKDPD
      | | | | | : | : : | : : : : : : : : : : | | | | : | |
Ma524.pep QQCGWLAHDFLHHQVFQDRFWGDLFGAFLGGVCQGFSSSSWWKDKHNTTHAAPNVHGEDPD
      170     180     190     200     210     220

      240     250     260     270     280
human  $\Delta 5$  INMHPFF---FALGKILSV---ELGKQKKKYPYNHQQHYFFLIGPPALLPLYFQWYIF
      | : | : : | : : : | : : : : | : : : | : | : | : | : | : |
Ma524.pep IDTHPLLWSEHALEMFSQVDEELTRMWSRFMVLN-QTWFFPILSFARLSWCLQSILF
      230     240     250     260     270     280

      290     300     310     320     329
human  $\Delta 5$  YFV-----IQKKWVDLAWMITFYVRFFLTYY--PLLGLKAFLGLFFIVRFL
      : : : : : | : : : : | : : : : : : : : : : : : : : : :
Ma524.pep VLPNGQAHKPSGARVPISLVEQLSLAMHWTWYLATMFLFIKDPV---NMLVYFLVSQAV
      290     300     310     320     330

      330     340     350     360     370     380
human  $\Delta 5$  ESMWFNTQMNHHPMHI---DHDRNMDWVSTQLLATCNVHKSANFNDWFSGHLNFQIEHH
      : : : : : | : : : : | : : : : : : : : : : : : : : : :
Ma524.pep CGNLLAFVFSLNHNGMPVISKEEAVDMDFFTKQIITGRDVHPGLFANWFTGGLNYQIEHH
      340     350     360     370     380     390

      390     400     410     420     430     440
human  $\Delta 5$  LFPTMFRHNYHKVAPLVQSLCAKRGIEYQSKPLLSAFADIHSLKESQQLWLDAYLHQX
      | | | : | : | : | : | : | : : : : : : : : : : : | : |
Ma524.pep LFPSMFPNHSKIQPAVETLCKKINVRVHTTGMEGTAEVFSPLNEVSKAASKMGKAQX
      400     410     420     430     440     450

```

Figure 31

37/40

# FastA Match of $\Delta 5$ -Desaturase (Ma29) and Human $\Delta 5$ -Desaturase

SCORES Init1: 145 Initn: 236 Opt: 266  
Smith-Waterman score: 400; 27.5% identity in 455 aa overlap

```

      10      20      30      40      50      60
human  $\Delta 5$  MAPDPVAAETAAGQPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPPGGSRVIS
      |||  |||:|:| :: :: ||| :|:|:|:| ||||| ::
Ma29.pep  MGTDOGKT---FTWEELAAHNTKDDLLLAIRGRVYDVTKFLSRHPGGVDTL
      10      20      30      40

      70      80      90      100     110
human  $\Delta 5$  HYAQDATDPFVAFHINKGLVKYMNSSLIGEL-SPEQPSF-EPTKNKELTDEFRELRT
      ||:|:| | :| | :| :: :| | | | | ||| :: |
Ma29.pep  LGAGRDVTFVFEMYHAF-GAADAIMKKYVGTLVSNELPIFPEPTVFHKTIKTRVEGYFT
50      60      70      80      90      100

120      130      140      150      160      170
human  $\Delta 5$  VERMGLMKANHVF--FLLYLLHILLDDGAAWLTWVFGTSFLPFLCAVLLSAVQAQAGW
: : : :: : | : : | : : | : : | : : | : : | : : |
Ma29.pep  DRNIDPKNRPEIWGRYALIFGSLIASYYAQLFVFPVVERTWLQVVF-AIIMGFACAQVGL
110     120     130     140     150     160

      180     190     200     210     220     230
human  $\Delta 5$  LQ-HDFGHLV-FSTSKWNHL--LHFEVIGHLKGAPASWNNMH-FQHHAKPNCFRKDPD
|| :|:| | : : | : | | :||| :| :| :| :| :|
Ma29.pep  NPLHDASHFSVTHNPTVWKILGATHDF----FNGASYLVWMYQHMLGHPYTNLAGADPD
170     180     190     200     210     220

      240     250     260     270     280
human  $\Delta 5$  INM-HPFFFALGKILSVELGKQKKKMPYNH--QHKYF-FLIGPPALLPLYFQWYIFYFV
: : : | :| :| :|:|:| || || :| | | :| :| :|:|
Ma29.pep  VSTSEP-----DVRRIKFNQKWF-VNHINQHMFPFLYGLLAFKVRIQDINILYFV
      230     240     250     260     270

      290     300     310     320     330
human  $\Delta 5$  IQ----RKKWVDLAWMITFY--VRFFLTYY--VPL--LGLKAFGLFFIVRFLESNWFVW
| : : : : | :| :| :| | | | | :| :| :| :| :|
Ma29.pep  KTNDAIRVNPISTWHTVMEWGGKAFFVWYRLIVPLQYLPGLKVLVLLFTVADMVSSYWLAL
280     290     300     310     320     330

      340     350     360     370     380     390
human  $\Delta 5$  VTQMNHIHPMHID---HFRN----MDWVSTQLLATCN-VHKSAFNDWFSGHLNFQIEHHLF
: | ||: :: :| | ||: | :| :| :| :| :| :| :| :|
Ma29.pep  TFQANHVVVEEVQWPLPDENGITCKDWAAMQVETTQDYAHDSLWTSITGSLNVAQVHHLF
340     350     360     370     380     390

390     400     410     420     430     440
human  $\Delta 5$  PTMPRHNHYHKVAPLVQSLCAKRGIEYQSKPLL-SAFADIHSLKESGQLWLDAYLHQX
|: :|:| : : :|:| :| :| :| :| :| :| :| :| :|
Ma29.pep  PAT/SQHHYPDILAIKNTCSETEVYPYLVKDTFWQAFASHLEHLRVGLRPPKEEX
400     410     420     430     440

```

Figure 32



38/40

Figure 33

Host(plasmid) Added substrate	334(pRAE-28-5) 25 $\mu$ M DGLA	334(pRAE-26-1) 25 $\mu$ M DGLA (g fatty acid/100 g fatty acid)	334(pRAE-33) 25 $\mu$ M DGLA 30°C/48 hrs lipid ( $\mu$ g)	334(pRAE-35) 25 $\mu$ M DGLA	334(pYX242) 25 $\mu$ M DGLA
Fatty acid					
C16:0	151.580	202.175	285.291	281.298	304.229
C16:1	406.279	485.631	552.951	569.298	608.123
C18:0	16.494	25.995	32.162	27.479	30.093
C18:1n-9	100.031	133.349	173.772	184.740	187.780
C18:2n-6		0.180			0.946
C18:3n-6		0.058	0.074		0.074
C20:0	3.844	4.205	7.118	7.285	6.288
C20:3n-6	96.576	118.657	134.859	139.292	125.448
C20:4n-6	(0.127%) 1.204	(0.075%) 0.878	(0.062%) 0.902	(0.063%) 0.927	(0.062%) 0.958
C22:0		0.150	0.119		0.125
C22:1n-9	0.162	0.139	0.299	0.275	0.392
Total Lipid	949.0	1169.0	1445.5	1468.0	1538.5

39/40

Figure 34a

Host(plasmid) Added substrate	334(pRAE-28-5) 25 $\mu$ M DGLA	334(pRAE-26-1) 25 $\mu$ M DGLA	334(pYX242) 25 $\mu$ M DGLA 30°C/48 hrs	334(pRAE-28-5) 25 $\mu$ M OA	334(pRAE-26-1) 25 $\mu$ M OA	334(pYX242) 25 $\mu$ M OA	334(pRAE-28-5) 25 $\mu$ M AA	334(pRAE-26-1) 25 $\mu$ M AA	334(pYX242) 25 $\mu$ M AA 30°C/48 hrs
Fatty acid		(g fatty acid/100 g fatty acid)	lipid ( $\mu$ g)						
C16:0	49.332	106.358	93.225	84.327	37.013	51.018	78.471	53.685	74.099
C16:1	141.178	256.622	277.028	269.009	107.066	172.485	230.45	141.526	181.298
C18:0	9.301	14.819	12.908	11.871	8.3	9.047	11.283	9.97	10.969
C18:1n-9	39.876	87.504	72.842	106.416	52.634	71.453	61.754	42.289	46.873
C18:2n-6				ND	ND	ND			
C18:3n-6									
C20:0	2.154	7.339			3.729			2.685	
C20:3n-6	45.395	56.346	55.306						
C20:4n-6	(0.106%) 0.412	(0.060%) 0.396	(0.065%) 0.402						
C20:5n-3									
Total Lipid	387	665	620	562	284	363	535	404	466
							(0.026%) 0.139	(0.019%) 0.077	(0.027%) 0.126

40/40

Figure 34b

Host(plasmid) Added substrate	334(pRAE-28-5) 25 $\mu$ M LA	334(pRAE-26-1) 25 $\mu$ M LA (g fatty acid/100 g fatty acid)	334(pYX242) 25 $\mu$ M LA 30°C/48 hrs fatty acid	lipid ( $\mu$ g)	334(pRAE-28-5) no substrate	334(pRAE-26-1) no substrate	334(pYX242) no substrate
Fatty acid							
C16:0	56.631	45.393		74.247	174.138	25.574	33.44
C16:1	181.311	117.045		208.029	277.122	43.193	47.189
C18:0	9.549	9.251		11.45	22.547	5.119	8.432
C18:1n-9	48.256	46.496		51.342	134.822	21.89	32.618
C18:2n-6	31.91	23.221		36.821			
C18:3n-6	(0.02%) 0.082	ND		(0.012%) 0.056			
C20:0		0.339			0.702		
C20:3n-6							
C20:4n-6							
C20:5n-3	0.121						
Total Lipid	407	279		460	746	127	168

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C12N 9/02, 15/64, 15/81, 15/82, C12P 7/64, C12N 5/16, A01K 67/00, A23L 1/00, A61K 31/202, 7/00</b>	<b>A3</b>	<b>(11) International Publication Number: WO 00/40705</b> <b>(43) International Publication Date: 13 July 2000 (13.07.00)</b>
<b>(21) International Application Number: PCT/US99/31163</b> <b>(22) International Filing Date: 29 December 1999 (29.12.99)</b>  <b>(30) Priority Data:</b> 09/227,613      8 January 1999 (08.01.99) <b>US</b>  <b>(71) Applicant: ABBOTT LABORATORIES [US/US];</b> CHAD-0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 6050 (US).  <b>(72) Inventors: MUKERJI, Pradip; 1069 Arcaro Drive, Gahanna, OH 43230 (US). LEONARD, Amanda, E., Y.; 581 Shadewood Court, Gahanna, OH 43230 (US). HUANG, Yung-Sheng; 2462 Danvers Court, Upper Arlington, OH 43220 (US). PARKER-BARNES, Jennifer, M.; 1311 Belcross Drive, New Albany, OH 43054 (US).</b>  <b>(74) Agents: BECKER, Cheryl, L. et al.; Abbott Laboratories, CHAD/0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-6050 (US).</b>	<b>(81) Designated States: AU, BG, BR, CA, CN, CZ, HU, IL, JP, KR, MX, NO, NZ, PL, RO, SI, SK, TR, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</b>  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 9 November 2000 (09.11.00)	
<b>(54) Title: HUMAN DESATURASE GENE AND USES THEREOF</b>  <b>(57) Abstract</b>  The subject invention relates to the identification of a gene involved in the desaturation of polyunsaturated fatty acids at carbon 5 (i.e., "human $\Delta 5$ -desaturase") and to uses thereof. In particular, human $\Delta 5$ -desaturase may be utilized, for example, in the conversion of dihomo- $\gamma$ - linolenic acid (DGLA) to arachidonic acid (AA) and in the conversion of 20:4n-3 to eicosapentaenoic acid (EPA). AA or polyunsaturated fatty acids produced therefrom may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Larvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

# INTERNATIONAL SEARCH REPORT

Inter. nal Application No

PCT/US 99/31163

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/02 C12N15/64 C12N15/81 C12N15/82 C12P7/64  
C12N5/16 A01K67/00 A23L1/00 A61K31/202 A61K7/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P A01K A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, BIOSIS, STRAND

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 46765 A (CALGENE LLC; ABBOTT LABORATORIES) 22 October 1998 (1998-10-22)  SEQ ID NO:24 and SEQ ID NO:34 page 65, line 10 - line 19 & WO 98 46763 A 22 October 1998 (1998-10-22) & WO 98 46764 A	1,3, 6-29, 31-56
X	LAMERDIN J.E. ET AL.: "BC269730_2." EMBL DATABASE ENTRY 060427; ACCESSION NO. 060427, 1 August 1998 (1998-08-01), XP002140846  --- -/--	6,7

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

23 June 2000

Date of mailing of the international search report

12/07/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Schönwasser, D

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/31163

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	CHO H. P. ET AL.: "Cloning, Expression, and Fatty Acid Regulation of the Human Delta-5 Desaturase" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 52, 24 December 1999 (1999-12-24), pages 37335-37339, XP002140847 ISSN: 0021-9258 figure 1 ---	1,3-22
E	WO 00 20603 A (ABBOTT LABORATORIES) 13 April 2000 (2000-04-13)  SEQ ID NO:24 and SEQ ID NO:31 ---	1,3, 6-29, 31-56
A	MICHAELSON L ET AL: "Isolation of a delta5-fatty acid desaturase gene from Mortierella alpina" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 30, 24 July 1998 (1998-07-24), pages 19055-19059, XP002076636 ISSN: 0021-9258 the whole document ---	1-56
A	KNUTZON D S ET AL: "Identification of Delta5 - desaturase from Mortierella alpina by heterologous expression in Bakers yeast and canola" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 45, 6 November 1998 (1998-11-06), pages 29360-29366, XP002106760 ISSN: 0021-9258 the whole document -----	1-56

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

## 1. Claims: 1-36,55,56

An isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence represented by SEQ ID NO:1; a purified protein encoded by said nucleotide sequence; a purified polypeptide which desaturates polyunsaturated fatty acids at carbon 5 and has at least about 50% amino acid similarity to the amino acid sequence of said purified protein; a method of producing a human delta5-desaturase comprising inter alia the step of isolating said nucleotide sequence represented by SEQ ID NO:1; a vector comprising a) a nucleotide sequence as represented by SEQ ID NO:1 operably linked to b) a promoter; a host cell comprising said vector; a plant cell, plant, or plant tissue comprising said vector; one or more plant oils or acids expressed by said plant cell, plant, or plant tissue; a transgenic plant comprising said vector; a mammalian cell comprising said vector; a transgenic, non-human mammal whose genome comprises a DNA sequence encoding a human delta5-desaturase operably linked to a promoter; a fluid produced by said transgenic, non-human mammal and a method for producing a polyunsaturated fatty acid comprising inter alia the step of isolating said nucleotide sequence represented by SEQ ID NO:1.

## 2. Claims: 37-45,54

A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to above mentioned method of producing a polyunsaturated fatty acid and a method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition in an amount sufficient to effect said treatment.

## 3. Claims: 46-48

A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to above mentioned method of producing a polyunsaturated fatty acid and 2) a pharmaceutically acceptable carrier.

## 4. Claims: 49-52



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to above mentioned method of producing a polyunsaturated fatty acid.

5. Claim : 53

A cosmetic comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to above mentioned method of producing a polyunsaturated fatty acid.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/31163

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9846765 A	22-10-1998	US 5972664 A	26-10-1999
		AU 7114798 A	11-11-1998
		AU 7114898 A	11-11-1998
		EP 0996732 A	03-05-2000
		EP 1007691 A	14-06-2000
		NO 994924 A	30-11-1999
		NO 994926 A	30-11-1999
		WO 9846764 A	22-10-1998
		US 6051754 A	18-04-2000
WO 0020603 A	13-04-2000	NONE	